A COMPARATIVE STUDY OF THE AEROBIC AND ANAEROBIC RESPIRATION IN THE STORAGE ORGANS OF DIFFERENT PLANTS

By

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INTRODUCTION

The anaerobic respiration of plants has been studied rather extensively. Every plant so far studied except a species of Elodea (Lyon, 1923) has been found capable of producing carbon dioxide in the absence of free oxygen. Because of this almost universal occurrence of the phenomenon, anaerobic respiration is now considered a normal process of plants and not an adaptation to an unusual condition. Alcoholic fermentation by yeast is a type of anaerobic respiration, and a similar process has been proved to occur in higher plants as a normal process and not as incidental to death. The present concept of the genetic connection of the anaerobic and aerobic phases of respiration will be discussed in the review of literature.

The chief purpose of the present study was to determine the ratio of the anaerobic to the aerobic respiration, the I/N value, for various plants and the effect of different treatments thereon. The literature contains conflicting data on this subject and further investigation was thought advisable.

Much of the work has been repetition of previous research, extending the experiments of earlier workers (Appleman, 1912, 1915, 1918; Bailey, 1921; Kimbrough, 1925, and others) to include anaerobic as well as aerobic respiration. The effects of various storage treatments, wounding, and treatment with ethyl bromide have been determined with potatoes. Carrots, parsnips, turnips, and onions have been subjected to different storage treatments in regard to temperature. Green sweet corn and tomato fruits have been studied at different stages of maturity. Mature corn grain, and soybeans have been studied at different moisture percentages.

REVIEW OF LITERATURE

William Cruikshank (Stiles and Leach, 1932) working with barley grain was first to notice the production of carbon dioxide in the absence of oxygen. In 1804, de Saussure observed that different green plants liberated carbon dioxide in an atmosphere of nitrogen. Much of the subsequent study of anaerobi respiration was with fruits. Berard (Hill, 1913) found that green fruits ripened in air but not in the absence of oxygen. Lechartier and Bellamy (1869) reported that apples evolved carbon dioxide for eight months, in the absence of oxygen, with the production of alcohol and carbon dioxide in almost equal proportions. When Pasteur (1872) showed that the ratio of carbon dioxide to alcohol was the same as in yeast fermentation, Lechartier and Bellamy (1872) repeated their work and found alcohol present deep in apple tissue where the presence of yeast was impossible. This was the first definite proof that anaerobic respiration is a normal, vital process in higher plants. Brefeld (1876) found that various molds, seeds, fleshy fruits, leaves, flowers, and wood gave off carbon dioxide without access to oxygen. None of the material used lived long in absence of oxygen except species of Mucor. Pfeffer (1878) was first to use the term intramolecular respiration for the process in plants by which food material is utilized by splitting of the molecules, with release of carbon dioxide, production of energy, and the formation of new products. He held the view that alcohol was an intermediate product of oxygen respiration and that the intramolecular phase preceded and caused the aerobic stage. Wortmann (1880) had the same general idea that Pfeffer held, but their theories have been superseded because experimental evidence failed to support them.

Böhm (1887) observed that the inner (anaerobic) respiration of potato tubers was independent of traumatic injury, but was greater in tubers sweetened by exposure to low temperature than in non-sweetened tubers.

Stich (1891) repeated some of Pfeffers work and found that the I/N value, ie. the ratio of anaerobic to aerobic respiration, varied with the stage of development of the material used. He concluded that the various chemical processes involved at different ages of plants affect the respiration differently. He also reported that, under certain conditions, equal amounts of carbon dioxide may be formed aerobically and anaerobically. He regarded the liberation of carbon dioxide as a link in a chain of reactions. Stich also found, with potato tubers, that wounding did not affect the anaerobic respiration; wounded tubers in hydrogen produced about as much carbon dioxide as unwounded tubers in air. Using gas mixtures, the respiratory quotient, R/Q, ie. the ratio between the carbon dioxide liberated and the oxygen absorbed, remained nearly constant until the oxygen dropped to 2 to 4 per cent.

Theoretically, anaerobic respiration should give rise to somewhat smaller amounts of carbon dioxide than aerobic respiration. In alcoholic fermentation, only one-third as much carbon dioxide is produced as when the same amount of sugar is completely oxidized aerobically. However, the I/N value has seldom been found to approximate the value 0.33, nor does it remain constant. Wilson's data, reprinted by Kostychev, (1927), contains I/N values from 0.18 - 1.20. Boysen-Jensen (1923) observed even greater variation in I/N values. Grape fruits gave very high I/N values, from 1.0 to 1.3. Potato tubers were more variable, giving values from 0.44 to 1.10. Kostychev (1927) states that Moeller also found variable I/N values. Richards (1896) found that carrots liberated about as much carbon dioxide in anaerobic as in aerobic conditions. Gustafson (1932) observed that the anaerobic respiration of cacti was about equal to the aerobic over a period of several days.

On the basis of the wide variation in I/N values, Moeller (Kostychev, 19

Borodin (Kostychev, 1927) found no increase in the aerobic respiration of an elder twig after a period of anaerobiosis, thereby questioning Pflügers (1875) theory of the formation of labile intermediate products by anaerobic respiration. Borodins conclusions have been disproved by workers (Stich, 1891; Gustafson, 1930, 1932) who have observed increased aerobic respiration in plant materials after a period of anaerobiosis. Gustafson (1930, 1932) has recently found that tomato fruits and branches of cacti liberate carbon dioxide more rapidly after removal from anaerobic conditions than did controls run continuously under aerobic conditions.

Diakonow (1886 et seq.) observed that certain common molds were incapable of life in the absence of oxygen when on a medium lacking saccharine nutrients, but readily produced carbon dioxide anaerobically on media containing sugars. Normal respiration occurred on sugar free media. He worked with some higher plants and found that seeds of Vicia faba produced more carbon dioxide in the absence than in the presence of oxygen, and that the seeds of Ricinus communis, very low in carbohydrates, showed a very low anaerobic respiration. Diakonow concluded from his experiments and those of others, that anaerobic respiration was not genetically connected with aerobic respiration. Palladin accepted Diakonow's conclusions for a time, and finally, Pfeffer gave up his view concerning the genetic connection of the two phases. From this general disbelief in the connection of the two phases, occurring about the end of the nineteenth century, the trend of thought has shifted, based on more recent work, until now the theory of genetic connection is almost universally accepted. Kostychev and his associates rank perhaps as the chief proponents of the newer theory, while Maquenne and Demoussy, (1921), and Boysen Jensen (1923) offer the strongest objections.

Buchner (1897) reported the presence of an enzyme, zymase, in yeast,

which could transform glucose into alcohol and carbon dioxide. This report stimulated much study . Godlewski and Polzeniusz, (1901, cited by Hill, 1913) discovered that, in the absence of oxygen, seeds gave rise to alcohol and carbon dioxide in about equal proportions, and they favored the theory that anaerobic respiration closely resembled alcoholic fermentation. Kostychev (1902) determined that anaerobic respiration can take place at the expense of different organic compounds. Tartrates, peptone, glycerin and other media were used. Aspergillus niger produced oxalic acid from sugars, but Mucor stotonifera produced oxalic acid in the presence of tartrates.

Nabokich (1903) also found different types of anaerobic respiration. Anaerobic respiration, or fermentation of hexose sugars, gave carbon dioxide and alcohol in almost theoretical proportions. Organic acids were utilized by some seeds in the absence of sugars, or other carbohydrates, with consequent reduction in the amount of alcohol produced. In lactic acid cultures, more carbon dioxide was formed than in alcoholic fermentation.

Stoklasa, Ernest, and Chocensky (1907) described an enzyme isolated from plants which resembled the zymasedescribed by Buchner, and which brought about lactic acid and alcoholic fermentation. These authors hypothesized the formation of alcohol in two steps: (1) the transformation of glucose to lactic acid by zymase, and (2) the production of alcohol and carbon dioxide from the lactic acid by the action of lactacidase.

Kostychev (1904) repeated Diakonow's work and found that previous errors in technique had given results which led to incorrect conclusions. Under proper conditions cultures of Rhizopus nigricans or Aspergillus niger produced carbon dioxide in the absence of oxygen when on media lacking sugar. After the data supporting Diakanow's conclusions had been proven

unreliable, and after the discovery of zymase in higher plants the general opinion again favored the theory of the connection of the two types of respiration.

Kostychev (1927) supports the theory of connection. He, with others mentioned, have found that alcohol is not always produced in anaerobic respiration, and it is now considered that a number of other products may arise by the anaerobic splitting of food materials. Acetaldehyde is one product, the existence of which has been proven. (Kostychev, 1912) Organic acids may be produced by anaerobic respiration (Nabokich, 1903).

Morkowin(1903) has shown for some plants that the I/N value for a given plant is not modified by stimulants. He used quinine hydrochloride, among others, and found that the anaerobic and aerobic phases of respiration were increased in the same proportion. Smirnoff (1903, cited by Kostychev, 1927) observed that wounding caused no change in the I/N value of the plants he used. His results do not agree with those of Stich (1891) and Richards (1896) who found that the I/N value was changed by wounding. Karlsen (1925) used ether and alcohol vapors on germinating wheat and found that the I/N ratio remained constant.

Against the theory of the connection of the two types of respiration, Maquenne and Demoussy (1921) found that certain foliage leaves were soon killed when oxygen was entirely withheld, but remained living in an oxygen supply far too low to support normal aerobic respiration.

Boysen-Jensen (1923) reported that the I/N values of some materials fell below 0.33. In many of the plants giving low values for I/N, the value decreased rapidly, perhaps due to injury of the plants by poisoning.

METHODS

Respiration

In all the experiments recorded in this paper, the rate of respiration was determined by measuring the amount of carbon dioxide liberated in unit time. The aerobic lots were kept in air, freed of carbon dioxide by passage through a tube containing moist soda lime, and a check bottle containing baryta water, before entering the respiration chamber. The anaerobic lots were in an atmosphere of nitrogen, probably with traces of other inert gases. The nitrogen used was water pumped, to eliminate possible contamination in oil-pumped nitrogen. It was purchased, compressed in tanks, from the Air Reduction Sales Company.

Traces of oxygen present in the nitrogen were removed by passing the gas over copper heated to about 400°C. in an electric combustion furnace. To insure greater surface area of copper for combining with oxygen, a quantity of granular cupric oxide was rolled up in a long tube of copper screen cloth, placed in a glass combustion tube, and reduced to metallic copper in a stream of hydrogen. The copper oxidized with use, and the reduction was repeated when necessary, usually daily. After passing over the copper, the nitrogen was drawn through a tube of soda lime, a bottle of alkaline pyrogallol, and another of baryta water to insure removal of carbon dioxide and traces of oxygen. The alkaline pyrogallol was made by dissolving 4 grams of pyrogallic acid in 10 ml. of water, and adding 40 ml. of potassium hydroxide solution (made by dissolving KOH in an equal weight of water). The potassium hydroxide solution was run under that of the pygallic acid. Mixing of the two solutions was effected by bubbling the nitrogen through the bottle. This method resulted in pyrogallol solutions of light red color which darkened very slowly with use. The solutions of pyrogallol were renewed

when they had darkened in color slightly.

The respiration apparatus was the one described by Kimbrough (1925) and Smith (1929), and was used very similarly. To more accurately control the gas flows through the different desiccators, a flow meter was used in each line. By means of screw clamps the rates of flow could be easily controlled. The flow was usually in the range of 2 to 5 liters of gas per hour, depending on the intensity of the respiration of the material used. The gas flow was kept as constant as possible when a series of experiments were to be compared.

A modification of Paladin's mercury regulator, as described by Kostychev, (1927), was used in the suction line to give a constant vacuum about 6 cm. of mercury under atmospheric pressure. Otherwise, a constant vacuum would have been impossible with the water aspirator because of fluctuating water pressure.

A water-filled U-tube was connected to the nitrogen line to release any pressure that might arise if the tank valve was opened too widely. Sufficient pressure, about 1 inch of water, was maintained on the nitrogen line to force the gas through the soda-lime tube. The flow from the pyrogallol bottle onward was due to suction.

The carbon dioxide was collected by passing the gas streams through Reiset towers, described by Gore (1911), filled with a solution of sodium hydroxide. Usually, the gas flows were stopped each day and the absorbing solutions renewed. Normal, or half normal sodium hydroxide was generally used, renewed each day, but with some plants, more concentrated solutions were used, or renewal made more frequently because of the large amounts of carbon dioxide produced.

About half an hour was necessary to replace the solutions. Appleman

(unpublished data) has found that stopping the gas flow for as much as an hour has no noticeable effect on the rate of respiration. If necessary, the copper oxide in the incoming nitrogen line was reduced at this same time, thereby preventing a second interruption of the gas flow.

The carbon dioxide was determined by a double titration using Küsters modification of Gore's (1911) method. An excess of solid barium chloride was added to the used alkali solutions which precipitated the carbon dioxide as barium carbonate. Normal hydrochloric acid was added until the solution was colorless, using phenolphthalein as an indicator. The solution was thoroughly stirred while adding the acid to prevent loss of carbon dioxide due to local concentrations of the acid in the solution. Methyl orange was added after the first end point had been reached and the titration continued until a bright pink color was reached. The amount of acid added between the first and second end points is considered equivalent to the carbon dioxide in the solution. One cubic centimeter of normal hydrochloric acid is equivalent to 0.022 gm. of carbon dioxide. Blank titrations were run on each set of stock solutions and correction made for the amount of carbonate present due to impurities in chemicals and contact with air. It is realized that the end points used are empirical, but according to Gore the results are closely accurate and are certainly reliable when used comparatively.

A refrigerator cooled by a Kelvinator unit was used to keep respiration chambers at a low temperature for certain experiments. The gas flows were controlled the same as in the warm chambers. The temperature fluctuated more widely in the cold chambers than in the warm ones. The warm chamber was nearly always within 0.2° C. of the set temperature; the cold chamber usually fluctuated over a range of 1.5° C. during a week, with even greater differences on week-ends when the laboratory was not heated.

Samples were run for a preliminary period, at the end of which the

the period varied with the material used. Material with thick tissues, eg. potato tubers, contain considerable carbon dioxide dissolved in the cell sap (Richards, 1896). The preliminary period allows this to escape. The air surrounding the anaerobic samples had to be replaced by nitrogen, and the oxygen dissolved in the plant sap allowed to diffuse out before true anaerobic conditions actually obtained within the tissue. Material from cold storage required several hours to reach a higher temperature. The preliminary period was kept very nearly constant in any series of experiments to be compared with each other.

Chemical Methods

SAMPLING:-- Material for sugar and moisture determinations was ground on a Nixtamal mill. After mixing the ground tissue thoroughly, sugar samples were weighed directly into counterpoised Kohhrausch flasks of 200 ml. capacity. Samples of potato tissue weighed 50 grams; of parsnip, carrot, and onion tissue, 25 grams; and of sweet corn, 16 grams. The action of enzymes was stopped immediately by the addition of 75 ml. of boiling 95 per cent alcohol to give a final concentration of 80 per cent after dilution by water present in the tissue.

MOISTURE:-- Samples for moisture determination were placed in tared watch-glasses with ground glass covers. These were weighed as soon as possible. The tissue was dried at 80° C. in a vacuum oven at a pressure about equal to 5 cms. of mercury until the weight was approximately constant. This usually required about 48 hours.

SUGAR DETERMINATION:-- The sugar samples were extracted by boiling 30 minutes on a steam bath in 50 - 60 per cent alcohol. After cooling, the samples

were made to volume with 95 per cent alcohol, and stored until analysed.

The extracted samples were filtered, using No. 1 Whatman paper. An aliquot of each filtrate, usually 150 or 125 ml. was evaporated almost to dryness on the steam bath to remove the alcohol. The residue was taken up in distilled water and the proteins and certain other organic materials were precipitated by the addition of saturated neutral lead acetate solution. The amount of lead acetate solution added varied with the material present, but was usually one to three milliliters. The excess lead was precipitated by the addition of an equal volume of 12 per cent sodium carbonate solution. After making to volume, both precipitates were removed by a single filtration through No. 1 Whatman paper. An aliquot of the filtrate, usually 50 ml. was used for reduction by the standard method of Munson and Walker (1906). The reducing sugars are reported as percentage of dextrose.

For the determination of total sugars, 5 ml. of concentrated hydrochloric acid were added to 50 ml. aliquots of the extract. After 24 hours for hydrolysis of sucrose to occur, the solution was made to volume, a portion neutralized with anhydrous sodium carbonate, and an aliquot used for a Munson-Walker reduction. The total sugars are expressed as percentage of invert sugar.

DETERMINATION OF POLYSACCHARIDES:-- Samples for the determination of starches and dextrins (soluble polysaccharides) were stored in cold alcohol, enough 95 per cent alcohol being used to give a final concentration of about 80 per cent. The material was dehydrated by the addition to each sample of three portions of 95 per cent alcohol at 24 hour intervals, decanting each time through a Buchner funnel fitted with No. 50 Whatman filter paper. After the last treatment with alcohol, all the material was transferred to the Buchner funnel, and washed with two portions of ether.

The dehydrated sample was then ground until all the material passed a 60 mesh sieve. Somewhat more than half the ground sample was weighed out for determination of dextrins, or soluble polysaccharides. The remainder was again ground until it nearly all would pass a 100 mesh sieve, then used for determination of starches plus dextrins. The starch content was determined by difference.

The soluble polysaccharides were removed by adding 300 ml. of water to each ground sample, and shaking constantly for one hour. The sample was then filtered through "Falten-filter" made by Carl, Schleicher, and Shull. To a 200 ml. aliquot of the filtrate, were added 12.5 ml. of concentrated hydrochloric acid (sp. gr. 1.18) and the mixture hydrolyzed 2.5 hours under a reflux condenser. After cooling, the sample was made to 500 ml. volume, mixing thoroughly. A portion of the solution was neutralized with anhydrous sodium carbonate, and a 50 ml. aliquot used for a standard Munson-Walker reduction. The dextrose value is multiplied by 0.9 to give a value for polysaccharides.

The samples for determination of total starches were each mixed with 150 ml. of water, and boiled 15 minutes, with stirring to gelatinize the starch. After cooling to 45° C., ten milliliters of filtered saliva previously diluted 1 : 10, were added to each sample. After digesting an hour at 45° C. the solutions were again brought to a boil, cooled, and saliva added as before. When the iodine test showed that the starch was completely digested, the solutions were made to volume in 250 ml. flasks, and then filtered. A 200 ml. aliquot of the filtrate was hydrolyzed with hydrochloric acid, the procedure from this stage being the same as described for dextrin analysis. The dextrose value is also multiplied by the factor 0.9 to give the starch value.

EXPERIMENTAL RESULTS

Potatoes (Solanum tuberosum L.)

Description of material

The experiments will be presented according to treatment and method used. In some cases, repetition was made in subsequent years with different material, so a general description of the material used will eliminate future repetition.

Some preliminary experiments were carried out on tubers purchased from a local farmer, November 2, 1931. He described them as the Green Mountain variety, but examination of the tubers indicated a mixture of varieties, or wide variance in tubers.

Tubers of the Russet Burbank variety were used for several experiments. These were purchased as needed from the local grocery. Different purchases were probably quite variable, but it is presumed that samples selected from a single purchase were quite uniform.

Maryland Certified Irish Cobbler seed potatoes were used during the winter of 1932-33. These were grown from certified seed produced in South Dakota and planted July 27, 1932. The grower was Mr. W. T. Pilchard, near Pocomoke, Md. The tubers were dug November 15, 1932, the tops having died naturally before digging. The tubers were delivered to the laboratory November 17, 1932, and stored until further treatment in wooden crates, protected from the light by a shade of black sateen cloth. For the cultural information concerning these tubers, and their delivery, the writer is indebted to Dr. Jehle of the Extension Service of the University of Maryland.

Maryland Certified McCormick potatoes were secured for experiments during the winter of 1933-34. These were grown near College Park as a 4-H project by the son of Dr. R. A. Jehle, and rogued by the latter during

the growing season. The tubers were from late planted seed, and were dug about the fourth of November. The tubers were stored in the basement of Dr. Jehle's residence until November 17, 1933 when they were delivered to the laboratory. All the tubers were washed, and rapidly dried on November 17. The samples were sorted out and stored November 20 and 21, 1934.

Later references will be made to a veriety by name only, without repetition of the above description.

Ethyl bromide treatment

Morkowin (1903) found that the I/N value was not changed by stimulants. Appleman (1915) has proved that treatment with ethyl bromide causes an increase in the catalase content and respiratory activity of potatoes. The treatment with ethyl bromide was carried out, therefore, to obtain additional information concerning the effect of stimulants on respiration.

Experiment 1.— The first series in this experiment was composed of four samples of Russet Burbank tubers bought locally June 15, 1932. The samples consisted of five tubers each and weighed from 841 to 880 grams. Two samples were untreated; the other two were placed in a desiccator of twelve liters capacity. Five milliliters of ethyl bromide were allowed to evaporate from a wad of cotton after placing the cover on the desiccator. The tubers were exposed to the gas one hour. All samples were then placed in the respiration chambers and twice subjected to full aspirator vacuum with slow release each time. The gas flows were steady for an additional 1.5 hours before beginning the record. The total preliminary period was about four hours. The data are given in table 1.

Table 1. Respiration of Russet Burbank potatoes at 30°C. after treatment with ethyl bromide. Begun June 22, 1932.

Respiration period	Carbon dioxide liberated per hour per kilo					
	controls			exposed 1 hr. to C_2H_5Br		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	12.4	10.5	.85	32.0	11.8	.37
2nd. day	12.1	6.2	.51	26.6	7.8	.29
3rd. day	13.0	6.5	.50	18.7	9.7	.52
4th. day	13.4	7.5	.56	16.9	11.4	.68
5th. day	15.6	9.2	.59	16.9	12.6	.75
6th. day	15.6	10.2	.66	16.4	13.6	.83
7th. day	17.8	10.4	.58	18.0	13.8	.77

Experiment 2.-- A second series on the effect of ethyl bromide treatment was run, using Irish Cobbler potatoes. The samples consisted of eight tubers each, and weighed from 954 to 981 grams. The treatment with ethyl bromide lasted one hour, but no vacuum was applied. The preliminary period extended for 17 hours. Samples were run at 22° C. and at 3½° C. No untreated controls were tested; compare with unstored tubers in table 27 which gives respiration of similar tubers just previous to this test.

Table 2. Respiration of Irish Cobbler potatoes after treatment with ethyl bromide. Begun December 6, 1932.

Respiration period	Carbon dioxide liberated per hour per Kilo at 22 ± .2° C			at 3 ± 1.0° C		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	15.9	7.7	.49	2.6	2.3	.90
2nd. day	12.1	5.3	.44	2.9	2.3	.77
3rd. day	11.4	5.2	.45	3.3	1.7	.52
4th. day	10.9	5.4	.49	3.3	1.4	.42
5th. day	9.2	5.3	.58	2.8	1.0	.35
6th. day	7.5	5.3	.71	3.1	.8	.26

This experiment shows that treatment with ethyl bromide gas stimulates the aerobic respiration of potato tubers more than it does the anaerobic. With the Russet Burbank potatoes, the high initial anaerobic respiration is apparent largely because the preliminary period was not long enough to allow complete displacement of all oxygen, and consequently, the values for the first day do not indicate true anaerobic respiration. There is no explanation known for the gradual increase in the respiration rates at the end of the experiment.

The anaerobic respiration of Irish Cobblers was very little stimulated

Storage in an air-tight container

Richards (1896) has reported the presence of large amounts of carbon dioxide present in the juice of potatoes and carrots, which was rapidly given off when the materials were heated. This experiment was designed to determine the effect of storage in a closed container, and the attendant increase in carbon dioxide content, on subsequent respiration.

The tubers used were of the Green Mountain variety. Four samples of ten tubers each were used, weighing from 1203 to 1244 grams each. Two lots were placed in a closed glass jar, the other two in test tube baskets. All four lots were stored one week in a refrigerator, the temperature of which dropped gradually from 40° to 36° F. at the end of the experiment. The results are given in table 3.

Table 3. Respiration at 22 ± .5° C of Green Mountain potato tubers after storage, at 40 to 36° F. Begun November 10, 1931.

Respiration period	Carbon dioxide liberated per hour per Kilo					
	After storage in air-tight vessel			After storage in air		
	Sample 1	Sample 2	Average	Sample 1	Sample 2	Average
	mg.	mg.	mg.	mg.	mg.	mg.
1st day	28.7	26.5	27.6	18.3	17.9	18.1
2nd day	19.5	18.5	19.0	16.5	15.6	16.1
3rd day	13.9	14.4	14.2	14.5	14.4	14.4
4th day	12.8	12.3	12.5	14.1	13.1	13.6
5th day	11.5	10.1	10.8	12.4	10.7	11.6

The results of this test indicate that storage in a closed container results in an increase in subsequent respiration. The effect is of brief duration, about two days, and may be largely due to the rapid release of carbon dioxide dissolved in the cell sap. The respiration shows some stimulus

due to the cold storage. This is one of the few experiments in which the variability of samples may be studied, and it is easily seen that agreement between samples is quite close in most instances.

Effect of storage in nitrogen

Irish Cobbler tubers were used for this experiment. The first series was run after seven days storage at 40 - 43° F., the second series after 21 days storage at the same temperature. The samples contained eight tubers each and weighed from 807 to 832 grams. All were selected from an unwashed lot of tubers after 54 days in room storage at about 65° F. (18° C.) average temperature. The control samples were stored in test tube baskets ; the samples which were stored in nitrogen were placed in glass jars, two samples in each, fitted with paraffined cork stoppers and sealed with melted paraffin. The stoppers were each fitted with two glass tubes closed with short lengths of rubber tubing and pinch clamps. One of the inlet tubes had a rubber tube attached to the inner end which extended to the bottom of the jar. After sealing a jar, the air was displaced by hydrogen, adding it at the top of the jar. The hydrogen was then displaced by adding nitrogen at the bottom of the jar. The nitrogen flow was continued for a few hours to wash out any additional air present. The clamps were then tightened to seal the jars, and the jars placed in storage. The jars contained as many tubers as possible. The only gas space was between tubers.

The preliminary periods were of about 19 hours duration. The chambers were at 22° C. The samples were transferred from storage jars to respiration chambers as quickly as possible but contact with the air was unavoidable. The results are given in tables 4 and 5.

Table 4. Respiration of Irish Cobbler potato tubers after seven days storage at 40 - 42° F. Begun January 17, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo.					
	After storage in air			After storage in Nitrogen		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	8.0	7.6	.95	9.2	7.8	.84
2nd. day	7.0	7.5	1.07	7.6	6.4	.84
3rd. day	6.6	5.9	.89	6.1	5.3	.86
4th. day	6.8	4.6	.68	5.9	4.8	.81
5th. day	5.9	4.9	.83	6.1	4.1	.68
6th. day	5.8	4.5	.77	4.5	4.3	.95

Table 5. Respiration of Irish Cobbler potato tubers after 21 days storage at 40 - 43° F. Begun January 30, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo.					
	After storage in air			After storage in Nitrogen		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	8.2	7.2	.88	14.0	11.5	.82
2nd. day	7.9	7.2	.91	10.9	8.6	.79
3rd. day	9.2	6.2	.67	11.1	6.2	.56
4th. day	9.3	4.8	.51	10.5	4.8	.46
5th. day	8.4	4.2	.50	9.5	4.7	.49
6th. day	8.5	4.3	.51	9.2	4.7	.51

Discussion

Storage in an atmosphere of nitrogen seems to stimulate the aerobic respiration slightly more than the anaerobic phase. The effect only lasts two or three days and is probably due to the rapid oxidation of intermediate

respiratory products formed during storage in nitrogen. The value for the first day's anaerobic run of the sample stored in nitrogen is rather high and may be due to liberation of dissolved carbon dioxide which had accumulated in the plant sap during the storage period.

Vacuum treatment

This experiment was carried out to find whether the rise in respiration after cold storage is due to a stimulating effect, or to the outward diffusion of carbon dioxide dissolved in the cold cell sap of the tubers.

Experiment 1.-- Four samples of Irish Cobbler tubers were used, each consisting of eight tubers and weighing approximately 1000 grams. These were selected from the general lot after 47 days storage at room temperature. Two samples were stored continuously in a refrigerator at about 2.2°C. (36° F.); the other two samples were stored 3 weeks in the refrigerator, then two weeks at room temperature. All samples were in wire baskets.

At the beginning of the respiration test, all samples were placed in the respiration chambers for four hours to allow the cold tubers to warm. One sample from each treatment was then subjected to full aspirator vacuum. One desiccator soon imploded resulting in a sudden release of the vacuum. It was replaced and the vacuum held at about one-third atmospheric pressure for an hour. The initial vacuum was not measured, but the aspirator was capable of reducing the pressure below 2 inches of mercury. After slow release of the vacuum, the gas flows were continued steadily until the end of the 17 hour preliminary period. The respiration test was begun February 6, 1933. See table 6.

Table 6. Respiration at 22° C. of Irish Cobbler potatoes, after different storage treatments, with and without evacuation.

Respiration period	Carbon dioxide liberated per hour per kilo					
	Tubers stored 5 weeks at 2.2° C.			Tubers stored 3 weeks at 2.2° C and 2 weeks at room temperature		
	evacuated	no vacuum	no vacuum / evacuated	evacuated	no vacuum	no vacuum / evacuated
	mg.		mg.	mg.	mg.	
1st day	17.7	22.5	1.27	6.6	6.5	.98
2nd day	20.8	22.9	1.10	6.8	6.6	.97
3rd day	18.0	19.2	1.07	6.5	6.4	.99
4th day	14.3	15.3	1.07	6.2	6.2	1.00
5th day	12.4	12.7	1.02	6.1	6.2	1.02
6th day	10.3	9.9	.97	5.8	6.1	1.06
7th day	8.7	8.4	.97	5.6	5.9	1.05

Experiment 2.--Similar results were gained from an experiment run on Green Mountain tubers after 59 days cold storage. Each lot consisted of nine tubers, and weighed 1000 to 1008 grams each. After storage one lot was subjected to aspirator vacuum three times, 30 minutes each, with an hour of steady flow after last evacuation. The respiration was all aerobic, at 22° C. The results are given in table 7. Respiration tests began January 1, 1932.

Table 7. Respiration at 22° C. of Green Mountain potatoes after 59 days cold storage.

Respiration period	Carbon dioxide liberated per hour per kilo of tubers		
	No vacuum	Vacuum	No vacuum / vacuum
	mg.	mg.	
1st. day	13.7	10.0	1.37
2nd. day	19.8	15.5	1.28
3rd. day	17.7	16.6	1.07
4th. day	18.6	17.1	1.09

The results of these tests indicate that the increase in the respiration of potatoes subsequent to a period of cold storage is due to a stimulating effect and not merely to the rapid loss of carbon dioxide from solution. This supports the conclusion of Kimbrough (1925) who found the respiratory quotient to remain about unity during the period of increased respiration.

The experiment shows that the stimulus due to cold storage disappears quickly; the tubers had a nearly constant respiration after two weeks in air subsequent to cold storage. If the vacuum treatment had any injurious effect, it was not evidenced by a significant rise in the respiration rate of the evacuated tubers stored at room temperature just preceding the experiment.

Wounding

There is much conflicting data on the effect of wounding on the respiration of potato tubers. Bohm (1887) found that wounding, or peeling, resulted in an increase in both the aerobic and anaerobic phases of respiration. Cold-sweetened tubers had a higher anaerobic respiration than unsweetened ones. Stich (1891) decided that the wounding of potatoes caused some stimulus in itself, but that most of the increased respiration was due to an increase in surface for oxygen absorption. The anaerobic respiration of wounded tubers was about the same as the aerobic respiration of normal tubers. Stich failed to regard this as an increase in anaerobic respiration, although the normal anaerobic respiration of potatoes is not equal to the aerobic. Richards (1896) found that respiration after wounding depended on the amount of tissue involved and the extent of wounding. He found that much carbon dioxide was stored in bulky tissue and released quickly after wounding. Johnstone (1925) concluded that most of the stimulus from wounding was due to the facilitation of gas exchange. Hopkins (1927) found an increase in the sugar content of tissue adjacent to wounds, and believed that a correlation existed between the sugar content and aerobic respiration. Lutman (1926) found that the anaerobic respiration of potatoes did not respond to wound stimulus.

Experiments on the effect of wounding were run on both Irish Cobbler and Russet Burbank varieties, both before and after cold storage.

The tubers were wounded by hacking with a light-weight, sharp butcher knife. The cuts were about one-eighth inch deep, and spaced a similar distance approximately. The cuts were made in two directions, so that they crossed each other at right angles, or nearly so. The wounded surface was approximately four times the area of the original surface of the tuber. The

tubers lost very little weight during the process, and almost never was a piece cut entirely free from the tuber.

Experiment 1.-- Russet Burbank potatoes were used in a number of tests. The respiration samples were small, consisting of 3 tubers each.

The first tests were on tubers purchased February 11. One series was run immediately, the other after 11 days storage at room temperature. The preliminary period was of about 2.5 hours duration. The results are given in table 8.

Table 8. Respiration of wounded Russet Burbank potatoes at 22° C. Purchased February 11, 1932.

Respiration period	Carbon dioxide liberated per hour per kilo					
	February 12-16, 1932			February 22-27, 1932		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st day	70.2	35.6	.51	39.7	25.3	.64
2nd day	72.5	28.6	.40	47.8	17.0	.35
3rd day	60.1	22.8	.38	42.9	14.4	.33
4th day	51.6	20.5	.40	35.1	10.7	.31
5th day				35.2	8.1	.23

Experiment 2.-- Russet Burbank tubers were used in this experiment to determine the effect of wounding on tubers after a long period of cold storage. The tubers were all stored at 2.2° C. (36° F.) from December 16, 1931 until date of test. The respiration was at 22° C. The preliminary periods were 3 hours time. Data are given in tables 9 and 10. The results of the series run March 9-12, are shown graphically in figure 1.

Table 9. Respiration of wounded Russet Burbank potatoes at 22° C. after storage at 2.2° C.

Respiration period	Carbon dioxide liberated per hour per kilo.					
	March 2-4, 1932			March 9-12, 1932		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	119.0	51.0	.43	123.7	44.4	.36
2nd. day	158.4	35.0	.22	151.8	33.8	.22
3rd. day				123.9	35.1	.28

Table 10. Respiration of wounded Russet Burbank potatoes after storage at 2.2° C. Values are averages of duplicates.

Respiration period	Carbon dioxide liberated per hour per kilo					
	April 2-4, 1932			April 7-10, 1932		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	119.5	34.4	.29	124.5	26.5	.21
2nd. day	159.8	29.7	.19	172.6	26.5	.15
3rd. day				123.1	24.6	.20

Experiment 3.-- The respiration samples of Irish Cobbler tubers consisted of eight tubers each, weighing about 900 grams. The respiration was at 22° C. The preliminary periods were of 17 to 18 hours duration. The storage period at 2.2° C. begun after 47 days storage at room temperature. The results are presented in table 11.

Table 11. Respiration of wounded and unwounded Irish Cobbler potato tubers at 22° C.

Storage period	temp	Respiration period	Carbon dioxide liberated per hour per kilo: Unwounded tubers: aerobic	anaerobic	I/N	Wounded tubers: aerobic	anaerobic	I/N
days	room		mg.	mg.		mg.	mg.	
47	temp	1st. day	5.1	6.3	1.23	72.2	14.0	.19
		2nd. day	5.5	3.9	.72	58.6	16.6*	.28*
56	2.2°C	1st day	28.7	12.6	.44	144.3	25.9	.18
		2nd day	29.5	12.1	.41	109.3	38.7*	.35*

* Indicates error due to growth of microorganisms.

Discussion

The most reliable control data for comparison of the I/N values of the Russet Burbank tubers are given in table 15. The tests were not made at the same time but a comparison of the I/N values is logical, and the wounded tubers have much lower I/N values. This indicates that wounding stimulates the aerobic respiration more than it does the anaerobic. The experiment with the Irish Cobbler tubers bears out this conclusion. The latter tubers were not washed before wounding and the growth of micro-organisms became noticeable the second day.

The wounded surfaces formed a new periderm in a week or less in air, but there was no trace of new periderm in the anaerobic samples. This confirms previous work. The great increase in aerobic respiration is partially due, perhaps, to the acceleration of metabolic activity in forming a new periderm.

The less pronounced increase in the anaerobic respiration may be due mainly to the increased surface for outward diffusion of gases.

Moist and dry storage

McCormick potatoes were used in this experiment. Two series were used, one stored 62 days, the other 90 days. Samples were of 8 tubers each, and weighed about 900 grams. Control samples were stored in wire test tube baskets protected from light by a screen of black cloth. The experimental samples were placed on moist sawdust in large flower pots, and covered with about 1.5 inches of the same. Water was added occasionally to keep the sawdust moist. The tubers were beginning to sprout in moist storage when the first series was run, January 22-28, 1934; when the second series was run, February 19-25, the tubers in the air stored samples had sprouts about .5 inch long, and those of the samples stored in sawdust were from 1 to 3 inches long. The sprouts were not removed before the respiration test. The respiration was at 22 ±.5° C.; the preliminary period lasted about 6 hours. The results are presented in tables 12 and 13.

Table 12. Respiration at 22 ± .5° C. of McCormick potatoes after storage in air, and in moist sawdust at room temperature for 62 days.

Respiration period	Carbon dioxide liberated per hour per kilo: After storage in air: aerobic	anaerobic	I/N	After storage in moist sawdust: aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	4.6	5.8	1.26	8.1	8.0	.99
2nd. day	5.2	5.4	1.03	7.6	5.8	.77
3rd. day	4.4	5.5	1.24	6.1	5.5	.90
4th. day	4.9	7.5 aerobic	1.55	6.7	6.5 aerobic	.97
5th. day	4.8	18.7	--	6.1	21.4	---
6th. day	4.9	37.7	--	6.1	34.5	---

Table 13. Respiration at 22 ± .5° C. of McCormick potatoes after storage in air, and in moist sawdust at room temperature for 90 days.

Respirations period	Carbon dioxide liberated per hour per kilo					
	After storage in air			After storage in moist sawdust		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	5.6	6.7	1.20	9.1	8.8	.96
2nd. day	5.7	5.4	.93	8.9	6.8	.76
3rd. day	5.7	6.3	1.11	8.5	5.3	.63
4th. day	5.4	6.6 aerobic	1.21	8.0	5.6 aerobic	.70
5th. day	5.2	19.4	---	7.6	21.1	---
6th. day	5.3	38.3	---	8.0	40.0	---

The results of this experiment show clearly that the aerobic respiration of potatoes is greater after storage in moist sawdust. Analyses, table 25, showed that the tubers stored in the moist sawdust contained more moisture and more sugars than those stored in the air. The anaerobic respiration showed very little stimulation as a result of moist storage; as a result the I/N values are consistently lower for the lots after moist storage.

Respiration subsequent to cold storage

The experiments herein reported were designed to extend previous observations on the respiration of potatoes. Kimbrough (1925) has made a thorough study of the respiratory response of potatoes to periods of cold storage and found a pronounced initial rise in the respiration rate when tubers were removed from a cold to a warm temperature. Böhm (1887), and Appleman (1915) have observed the same effect. Böhm reported that the anaerobic respiration was also increased after exposure to cold. Müller-Thurgau (1882), Appleman (1912), and Hopkins (1924) have found that the sugar content of potatoes increases greatly during cold storage. A temperature of 2° C. (36° F.) or less is necessary to cause large increases in the sugar content, or a pronounced increase in the respiratory rate. Barker (1933) stored tubers at temperatures of - 1° C. and 1° C. and found that, after a brief initial rise, the respiration rate was below normal and he presents as explanation the hypothesis that a depressant is formed, or initiated, at low temperatures which later affects the respiration. His data are different from those of the other workers mentioned.

Experiment 1.-- The preliminary tests were made with Green Mountain tubers. The samples consisted of ten tubers each, and weighed from 1097 to 1164.5 grams when stored. The respiration was at 22° C. The record was begun after preliminary periods of about 4 hours.

The cold storage temperature was between 36° F. and 41° F. (2.2-5.0° C.) the average being much nearer the minimum. Three tests were run after different periods of storage. The records are presented in table 14.

Table 14. Respiration at 22° C. of Green Mountain potatoes after cold storage.

Storage		Respiration period	Carbon dioxide liberated per hour per kilo		
Period	Temp.		aerobic	anaerobic	I/N
days			mg.	mg.	
21	2.2-4.5° C (36-40°F)	1st. day	18.3	15.3	.83
		2nd. day	22.8	13.7	.60
		3rd. day	21.9	12.2	.56
		4th. day	20.2	11.4	.57
		5th. day	18.0	11.6	.64
		6th. day	15.6	14.8	.96
		7th. day	15.3	16.0	1.05
31	2.2-4.5°C (36-40°F)	1st. day	16.4	14.1	.86
		2nd. day	23.9	13.2	.55
		3rd. day	28.8	14.9	.52
		4th. day	23.8	12.1	.51
		5th. day	21.7	11.4	.52
		6th. day	20.0	11.0	.55
		7th. day	17.8	10.7	.60
42	2.2-5.0°C (36-41°F.)	1st. day	19.7	15.1	.76
		2nd. day	23.7	12.2	.51
		3rd. day	20.4	13.7	.67
		4th. day	19.4	13.8	.71
		5th. day	18.5	13.2	.71

Experiment 2.-- Russet Burbank potatoes purchased December 15, 1931 were used in this experiment. The storage temperature was more constant than in Experiment 1, and averaged a little lower. The samples contained eight tubers each and weighed about 1100 grams. The respiration was at 22° C. The preliminary period was 2 hours for the samples stored 25 days, and about 4 hours for the samples stored 35 days. The results of both series are given in table 15.

Table 15. Respiration at 22° C. of Russet Burbank potatoes after cold storage.

Storage		Respiration period	Carbon dioxide liberated per hour per kilo		
Period	Temp.		aerobic	anaerobic	I/N
Days			mg.	mg.	
25	2.2° C. (36° F.)	1st. day	25.2	16.6	.66
		2nd. day	33.6	12.6	.37
		3rd. day	35.8	12.2	.34
		4th. day	29.9	10.2	.34
		5th. day	23.8	8.6	.36
		6th. day	17.7	7.6	.43
		7th. day	12.2	7.3	.60
35	2.2-2.8° C. (36-37° F.)	1st. day	21.2	14.5	.68
		2nd. day	26.7	12.3	.46
		3rd. day	24.5	11.6	.47
		4th. day	21.6	11.9	.55
		5th. day	15.9	11.3	.71
		6th. day	11.6	10.1	.87
		7th. day	9.2	9.3	1.01

Experiment 3.-- The experiment on the effect of cold storage was repeated with Irish Cobbler tubers. These samples were selected from the general lot after 47 days storage in the laboratory at a mean temperature of about 65° F. The samples weighed about 1000 grams each and consisted of eight tubers. Storage at 36° F. was begun January 2, 1933; the respiration test was begun January 23; the preliminary period lasted 18.5 hours. The results are given in table 16.

Table 16. Respiration of Irish Cobbler potato tubers at high and low temperatures after 21 days storage at 36° F. (2.2° C.)

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 22±.2° C.			at 2.5±.5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	12.6	13.5	1.07	3.9	3.1	.81
2nd. day	11.8	10.9	.93	3.7	3.0	.81
3rd. day	10.7	8.8	.82	3.4	2.4	.71
4th. day	12.6	8.6	.68	3.3	2.6	.79
5th. day	10.1	9.1*	.90	2.9	1.7	.59
6th. day	9.1	9.9*	1.09	2.4	1.4	.56

* Rise due to unknown cause; breakdown of tissue not evident.

Experiment 4.-- Two series were run in this experiment, one after 21 days storage at 36-38° F., the other after 56 days storage. The tubers used were of the Irish Cobbler variety and had been stored at room temperature 47 days before beginning the storage at low temperature. The samples consisted of eight tubers each and weighed about 1000 grams. The respiration chambers were at 22° C. The preliminary period lasted about 18 hours. The results are presented in table 17, and also in graph, figure 2.

Table 17. Respiration at 22° C. of Irish Cobbler potatoes after storage at 2.2-3.3° C. (36-38° F.)

Storage		Respiration period	Carbon dioxide liberated per hour per kilo		
Date	Period		aerobic	anaerobic	I/N
	days		mg.	mg.	
Jan. 2, to Jan. 23.	21	1st. day	12.6	13.5	1.07
		2nd. day	11.8	10.9	.93
		3rd. day	10.7	8.8	.82
		4th. day	12.6	8.6	.68
Jan. 2, to Feb. 27.	56	1st. day	28.7	12.6	.44
		2nd. day	29.5	12.1	.41
		3rd. day	24.8	11.1	.45
		4th. day	20.2	11.6	.58
		5th. day	15.8	11.2	.71

Experiment 5.-- Tubers of the McCormick variety were used in this experiment. Samples were selected about two weeks after the potatoes were harvested; each contained 8 tubers and weighed about 900 grams. One series of samples was stored at 22 ± .5° C. in the respiration thermostat, the other series in the refrigerator at 1.5 ± .5° C. These were removed after different periods of time to 22° C. and the respiration compared with tubers kept constantly at 22° C. Due to shortage of material, the last two samples were taken from room temperature storage and kept at 22° C. for 28 days preceding the respiration test. These last samples are compared with samples stored 76 days at the low temperature. The results of this series of tests are presented in tables 18, 19, 20, and 21 and in figure 3.

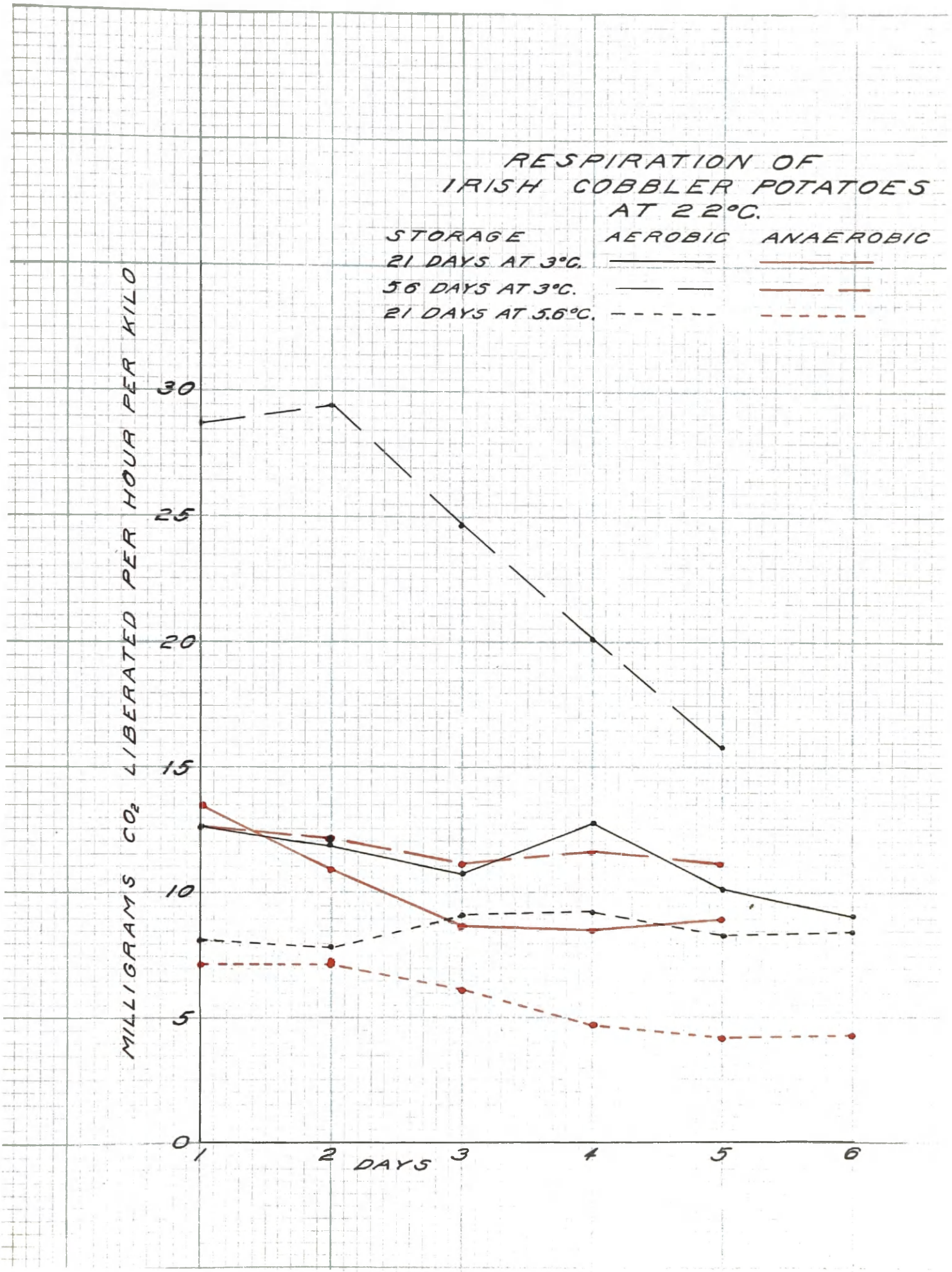

RESPIRATION OF
IRISH COBBLER POTATOES
AT 22°C.
STORAGE
AEROBIC
ANAEROBIC
21 DAYS AT 3°C.
56 DAYS AT 3°C.
21 DAYS AT 5.6°C.
MILLIGRAMS CO_2 LIBERATED PER HOUR PER KILO
30
25
20
15
10
5
0
1
2
DAYS
3
4
5
6

FIG. 2.

Table 18. Respiration at 22° C. of McCormick potatoes after about 2 weeks cellar storage. November 20-27, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo		
	aerobic	anaerobic	I/N
	mg.	mg.	
1st. day	29.8	16.8	.56
2nd. day	23.5	14.6	.62
3rd. day	20.3	12.4	.61
4th. day	16.7	40.8 aerobic	---
5th. day	15.1	36.7	---
6th. day	15.3	19.8	---
7th. day	12.9	16.3	---

Table 19. Respiration at 22° C. of McCormick potatoes after warm and cold storage for 21 days. Test, December 11-18, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					
	After storage at 22 ± .5° C.			After storage at 1.5 ± .5° C		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	6.4	8.1	1.26	29.3	23.6	.80
2nd. day	6.3	7.5	1.18	28.5	23.1	.81
3rd. day	6.5	9.2	1.42	23.3	18.9	.81
4th. day	6.1	7.8	1.28	18.1	17.3	.96
		aerobic			aerobic	
5th. day	5.9	24.6	----	16.4	40.4	---
6th. day	6.2	43.2	----	15.0	41.5	---
7th. day	6.1	30.0	----	13.6	25.6	---

Table 20. Respiration at 22° C. of McCormick potatoes after warm and cold storage for 49 days. Test, January 8-14, 1934.

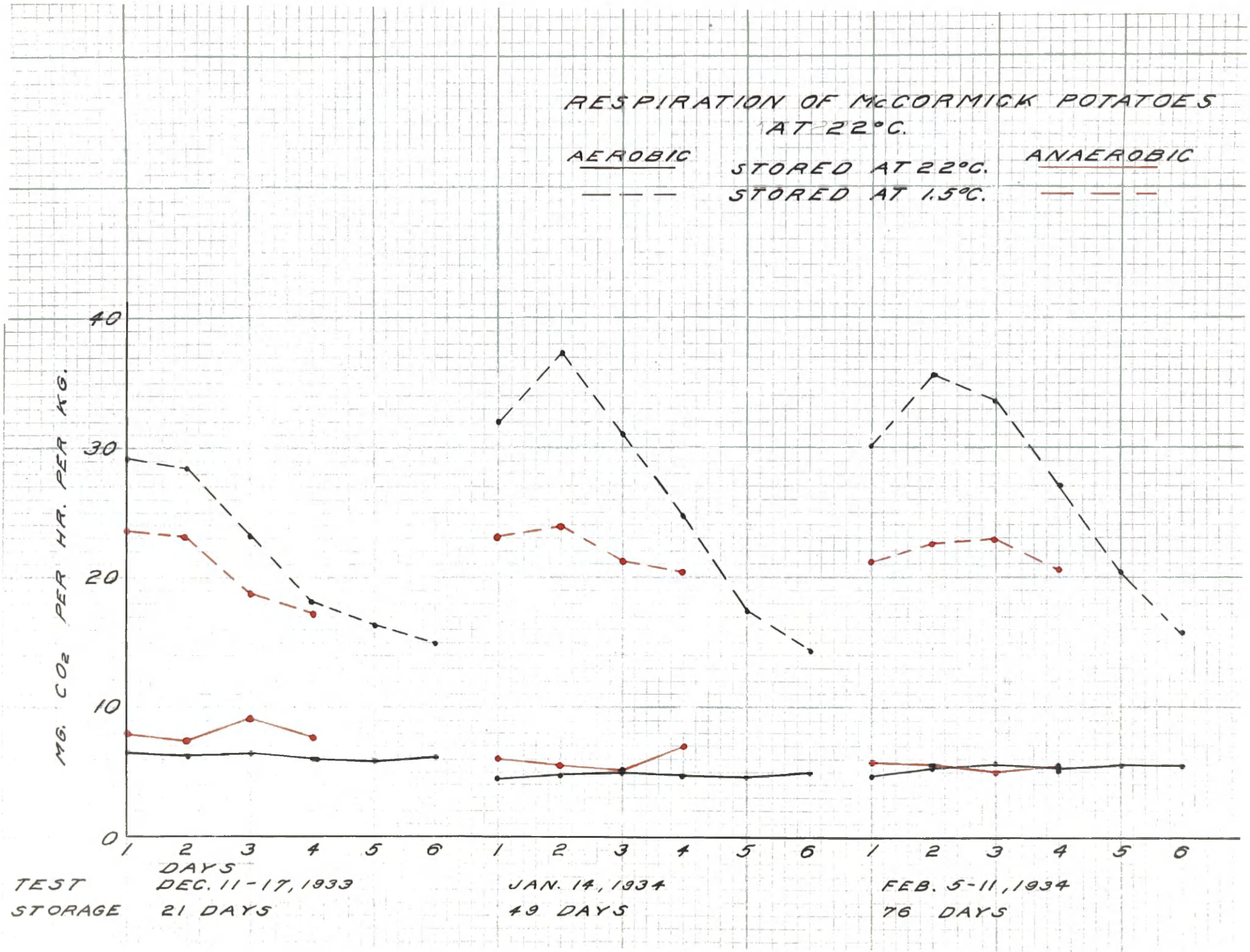
Respiration period	Carbon dioxide liberated per hour per kilo					
	After storage at 22 ± .5° C			After storage at 1 ± .5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	4.5	6.1	1.35	32.0	23.1	.72
2nd. day	4.9	5.6	1.14	37.4	24.0	.64
3rd. day	5.0	5.2	1.03	31.0	21.3	.69
4th. day	4.8	7.1	1.47	24.7	20.4	.83
		aerobic			aerobic	
5th. day	4.8	19.6	---	17.5	40.1	---
6th. day	5.0	36.4	---	14.5	43.2	---

Table 21. Respiration at 22° C. of McCormick potatoes after warm and cold storage for 76 days. Test, February 5-12, 1934.

Respiration period	Carbon dioxide liberated per hour per kilo					
	After storage at 22 ± .5° C.*			After storage at 1 ± .5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	4.7	5.9	1.24	30.1	21.1	.70
2nd. day	5.5	5.6	1.01	35.7	22.6	.63
3rd. day	5.6	5.1	.90	33.7	23.0	.68
4th. day	5.4	5.5	1.02	27.0	20.6	.76
		aerobic			aerobic	
5th. day	5.6	11.1	----	20.2	41.7	---
6th. day	5.6	34.5	----	15.7	42.9	---

* Storage at room temperature for 49 days; at 22° C. for last 28 days.

FIG. 3.


RESPIRATION OF McCORMICK POTATOES AT 22°C.
AEROBIC
STORED AT 22°C.
STORED AT 1.5°C.
ANAEROBIC
MG. CO2 PER HR. PER KG.
0
10
20
30
40
1 2 3 4 5 6
DAYS
TEST
STORAGE
DEC. 11-17, 1933
21 DAYS
JAN. 14, 1934
49 DAYS
FEB. 5-11, 1934
76 DAYS

Experiment 6.-- Two series of Irish Cobbler tubers were run after 7 and 21 days storage, respectively, at 4.5-6.0° C. (40-43° F.) Each sample contained eight tubers and weighed about 850 grams. The tubers had been stored at room temperature for 54 days before the cold storage was begun. The respiration chambers were at 22° C. The preliminary period lasted about 18 hours. The results are presented in table 22. Storage was begun January 9, 1933.

Table 22. Respiration at 22° C. of Irish Cobbler potatoes after storage at 4.5-6.0° C. (40-43° F.).

Storage		Respiration period	Carbon dioxide liberated per hour per kilo		
Date	Period		aerobic	anaerobic	I/N
	days		mg.	mg.	
		1st. day	8.0	7.6	.95
		2nd. day	7.0	7.5	1.07
Jan. 9 -		3rd. day	6.6	5.9	.89
to	7				
Jan. 16.		4th. day	6.8	4.6	.68
		5th. day	5.9	4.9	.83
		6th. day	5.8	4.4	.77
		1st. day	8.2	7.2	.88
		2nd. day	7.9	7.2	.91
Jan. 9 -		3rd. day	9.2	6.2	.67
to	21				
Jan. 30.		4th. day	9.3	4.8	.51
		5th. day	8.4	4.2	.50
		6th. day	8.5	4.3	.51

Experiment 7.-- Three lots of McCormick potatoes were stored at 6.5 ± 2.0° C. on November 21, 1933. The potatoes had been harvested about November 4, and stored at approximately 45° F. until start of experiment. Each sample

consisted of 8 tubers and weighed about 900 grams. The lots were stored 53, 83, and 111 days respectively. At the end of a storage period the two samples of a lot were removed, and placed in the respiration chambers at 22 .5° C., one sample under aerobic, the other under anaerobic conditions. The preliminary periods were of about 5.5 hours duration. The results are presented in table 23 and in figure 4.

Table 23. The respiration at 22° C. of McCormick potatoes after storage at 6.5 ± 2° C.

Storage		Respiration	Carbon dioxide liberated per hour per kilo		
Date	Period		aerobic	anaerobic	I/N
	days		mg.	mg.	
Nov. 21, 1933 to Jan. 15, 1934	53	1st. day	17.5	17.7	1.01
		2nd. day	18.2	17.6	.97
		3rd. day	14.9	15.2	1.02
		4th. day	11.5	12.6 aerobic	1.10
		5th. day	10.0	37.0	----
Nov. 21, 1933 to Feb. 12, 1934	83	1st. day	17.8	15.4	.86
		2nd. day	19.3	16.2	.84
		3rd. day	14.3	13.0	.91
		4th. day	12.8	11.5	.91
		5th. day	10.8	11.2	1.04
		6th. day	9.7	39.0 aerobic	----
Nov. 21, 1933 to Mar. 12, 1934	111	1st. day	13.9	13.2	.95
		2nd. day	16.4	14.4	.89
		3rd. day	13.5	11.2	.83
		4th. day	9.8	9.4	.96
		5th. day	9.7	32.2 aerobic	---

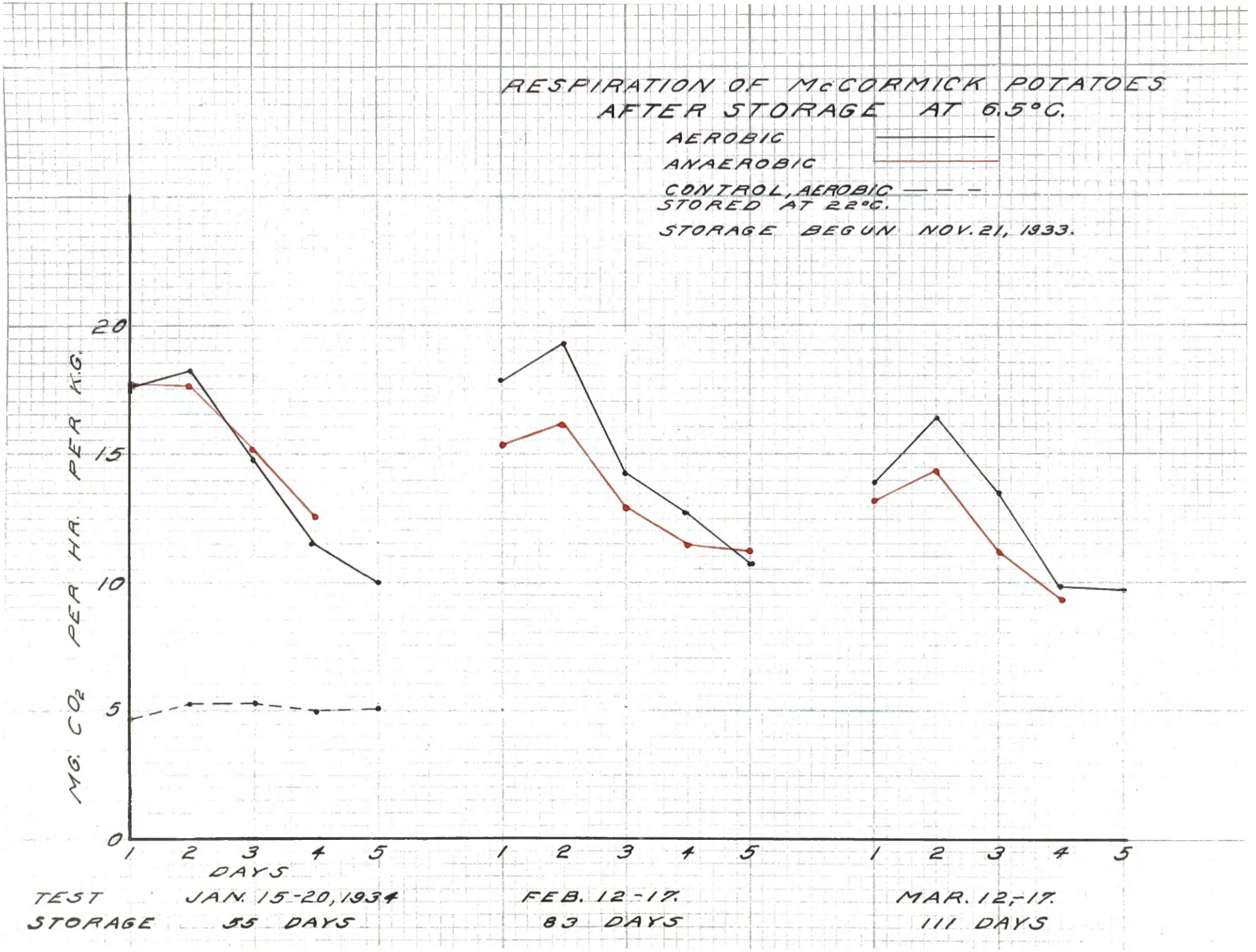

RESPIRATION OF McCORMICK POTATOES
AFTER STORAGE AT 6.5°C.
AEROBIC
ANAEROBIC
CONTROL, AEROBIC
STORED AT 22°C.
STORAGE BEGUN NOV. 21, 1933.
MG. CO2 PER HR. PER K.G.
0
5
10
15
20
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
DAYS
TEST
STORAGE
JAN. 15-20, 1934
55 DAYS
FEB. 12-17.
83 DAYS
MAR. 12,-17.
111 DAYS

FIG. 4.

Experiment 8.-- In order to study the effect of long storage at low temperature on respiration, two samples were placed at the cold temperature, 1.5 ± 1° C. as soon as received, and tested at intervals. The anaerobic sample was stored in air during the interval between respiration tests. The data are presented in table 24.

Table 24. Respiration of McCormick potato tubers kept constantly cold, at 1.5 ± 1° C. (35 ± 2° F.)

Date	Respiration period	Carbon dioxide liberated per hour per kilo		
		aerobic	anaerobic	I/N
		mg.	mg.	
Nov. 20-28, 1933	1st. day	7.1	5.7	.80
	2nd. day	5.3	4.1	.79
	3rd. day	5.8	3.0	.52
	4th. day	5.6	2.1	.37
	5th. day	6.0	2.0	.33
	6th. day	6.4	1.2	.19
	7th. day	5.2	2.4	.46
Jan. 15-20, 1934	1st. day	4.9	4.5	.91
	2nd. day	4.6	4.8	1.04
	3rd. day	5.3	3.9	.73
	4th. day	5.0	3.1	.63
	5th. day	5.1	3.2	.64
Feb. 20-25, 1934	1st. day	3.1	3.8	1.24
	2nd. day	3.2	4.4	1.40
	3rd. day	3.6	4.4	1.20
	4th. day	3.4	3.2	.95
	5th. day	3.3	2.6	.78
	6th. day	3.2	2.8	.87

Table 25. Sugar content of McCormick potatoes after different storage treatments.

Storage Time	Temp.	Moisture	Reducing sugars as dextrose		Total sugars as invert	
			wet wt.	dry wt.	wet wt.	dry wt.
		per cent	per cent	per cent	per cent	per cent
Original sample		77.25	.98	4.32	1.31	5.75
Nov. 21-Dec. 11	22° C	75.80	.80	3.31	1.26	5.20
	1.5° C	78.95	1.53	7.28	1.97	9.35
Nov. 21-Jan. 8	22° C	75.77	.35	1.46	.68	2.81
	1.5° C	76.40	1.86	7.87	2.39	10.11
Nov. 21-Jan. 15	6.5° C	77.17	1.01	4.44	1.20	5.26
Nov. 21-Jan. 22	Room air	76.03	.45	1.88	.68	2.82
	Room sawdust	77.75	.62	2.77	.86	3.86
Nov. 21-Feb. 5	22° C	75.33	.19	.75	.37	1.50
	1.5° C	75.55	1.56	6.38	2.22	9.09
Nov. 21-Jan. 8 Jan. 8 -Feb. 5	Room air: 22° C	76.38	.29	1.24	.48	2.03
Nov. 21-Feb. 12	6.5° C	76.39	.75	3.19	.90	3.82
Nov. 21-Feb. 19	Room air	76.67	.24	1.01	.42	1.81
	Room sawdust	81.02	.66	3.49	.84	4.41
Nov. 21-Mar. 12	6.5° C	75.48	.51	2.10	.68	2.77

Discussion

The results of the above experiments show that a period of cold storage results in an initial increase in the subsequent respiration at a higher temperature. It is clearly evident that this initial increase is more

pronounced in the aerobic phase than in the anaerobic phase of respiration.

All varieties used show a large initial increase in respiration after storage at 2 - 3° C. (36-38° F.). A period of 21 days was not long enough to cause much stimulus in Irish Cobbler tubers.

The tubers stored at about 6.5° C. (40-45° F.) showed some initial increase in respiration when changed to a warm temperature.

There seems to be a correlation between the sugar content, and the respiratory response to cold storage. It is shown in table 25 that the greatest increase in sugar content occurred at the lowest temperature used, 2 - 3° C. and that a smaller increase developed at 6.5° C. No development of a depressant as mentioned by Barker (1933) was noticed. In the six days that the experiments endured, the respiration of cold stored lots did not decrease to that of unstored controls, where Barker found a decrease to a rate below that of normal tubers.

While not shown directly in any table or figure, it is of interest to subtract the values for the anaerobic respiration from those for the aerobic. When these differences are plotted, the curve shows an initial increase. This offers most positive evidence that the initial increase in respiration is actually due to oxidation processes, and not alone to liberation of carbon dioxide physically bound. If the latter condition were the case, similar amounts of carbon dioxide would be liberated in both aerobic and anaerobic samples, and the difference in the amount of carbon dioxide produced would remain almost constant.

The significant result of the experiment is the fact that in potatoes the anaerobic phase of respiration does not show as definite initial increase after cold storage as does the aerobic.

In experiment 8, (tab. 24), it is shown that the anaerobic respiration rates of tubers at low temperatures increases after a period of storage. Samples were stored several weeks at 2° C. and the respiration rates determined at the same temperature. The rate of aerobic respiration decreased steadily for several weeks. The anaerobic respiration decreased to a very low rate at the end of the first week of cold storage, but after several weeks, it was greater, and the I/N values about the same as for samples stored at warm temperature.

Respiration at high and low temperatures

Previous experiments indicated that the I/N values were not the same for potatoes respiring at different temperatures. The following experiments were planned to give more definite data on this point.

Experiment 1.— Russet Burbank tubers, purchased October 29, 1932, were used for this experiment. The samples consisted of nine tubers each and weighed from 1518 to 1589 grams. Respiration tests were made at 22 ± .2° C. and at 2.5 ± .5° C. The results are presented in table 26.

Table 26. Respiration rates of Russet Burbank potato tubers at high and low temperatures.

Respiration period	Carbon dioxide liberated per hour per kilo of tubers					
	at 22° C.			at 2.5 ± .5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	10.1	11.0	1.08	3.2	3.2	1.01
2nd. day	9.9	8.9	.90	2.9	2.7	.93
3rd. day	9.4	6.5	.70	2.6	2.3	.88
4th. day	8.8	(7.1)*	(.81)	2.9	1.9	.66
5th. day	8.4	(9.5)	(1.13)	3.1	1.9	.62
6th. day	8.6	(11.9)	(1.39)	3.4	1.8	.54
7th. day	8.0	(12.8)	(1.59)	3.5	1.5	.44

* Values in parentheses probably abnormal due to injury resulting from anaerobiosis.

The tubers run anaerobically at 22° C. had a sour odor at the end of the experiment, but showed no visible signs of injury. Some injury evidently occurred because the tubers were kept several months and never recovered. They formed fewer sprouts, lost more weight and had discolored core tissue

in comparison with the normal aerobic sample.

Three series of Irish Cobbler tubers were tested. Each lot consisted of eight tubers and weighed about 1000 grams.

Experiment 2.-- Three series of samples of Irish Cobbler tubers were used in this experiment, one immediately after the potatoes were harvested, the others after 54 and 96 days storage at room temperature. The samples consisted of 8 tubers each; the samples of the first series weighed about 1150 grams each; those of the other series weighed about 850 grams. The respiration tests were made at 22 ±.2° C. and at 2.5 ±.5° C. The preliminary periods were of about 18 hours duration. The results of this experiment are presented in table 27, and in figure 5.

Table 27. Respiration of Irish Cobbler potatoes at high and low temperatures after storage at room temperature, mean about 18° C. (65° F.)

Storage period	Respiration period	Carbon dioxide liberated per hour per kilo at 22 ± .2° C			at 2.5° ± .5° C.		
		aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
days		mg.	mg.		mg.	mg.	
0	1st. day	12.1	11.1	.92	4.6	4.0	.86
	2nd. day	11.5	10.1	.88	3.9	2.9	.74
	3rd. day	10.5	9.4	.90	4.2	1.9	.44
	4th. day	9.6	9.5	1.00	4.7	1.5	.33
	5th. day	8.9	7.8	.87	4.9	1.3	.27
	6th. day	8.4		---	4.6	1.2	.27
	7th. day	8.1		---	4.5	1.2	.26
54	1st. day	5.0	6.0	1.20	1.5	1.2	.84
	2nd. day	5.0	3.5	.71	1.5	1.8	1.22
	3rd. day	4.8	3.1	.64	1.4	1.4	.97
	4th. day	4.8	3.2	.65	2.1	1.2	.61
	5th. day	5.0	3.3	.66	1.9	1.0	.54
	6th. day	4.6	3.5	.77	2.2	.8	.38
96	1st. day	6.3	5.2	.81	1.8	1.5	.82
	2nd. day	5.7	3.2	.56	2.0	1.7	.88
	3rd. day	6.2	2.7	.43	2.1	1.3	.64
	4th. day	7.1	2.6	.36	2.1	1.2	.58
	5th. day	7.3	2.5	.34	2.1	.8	.37

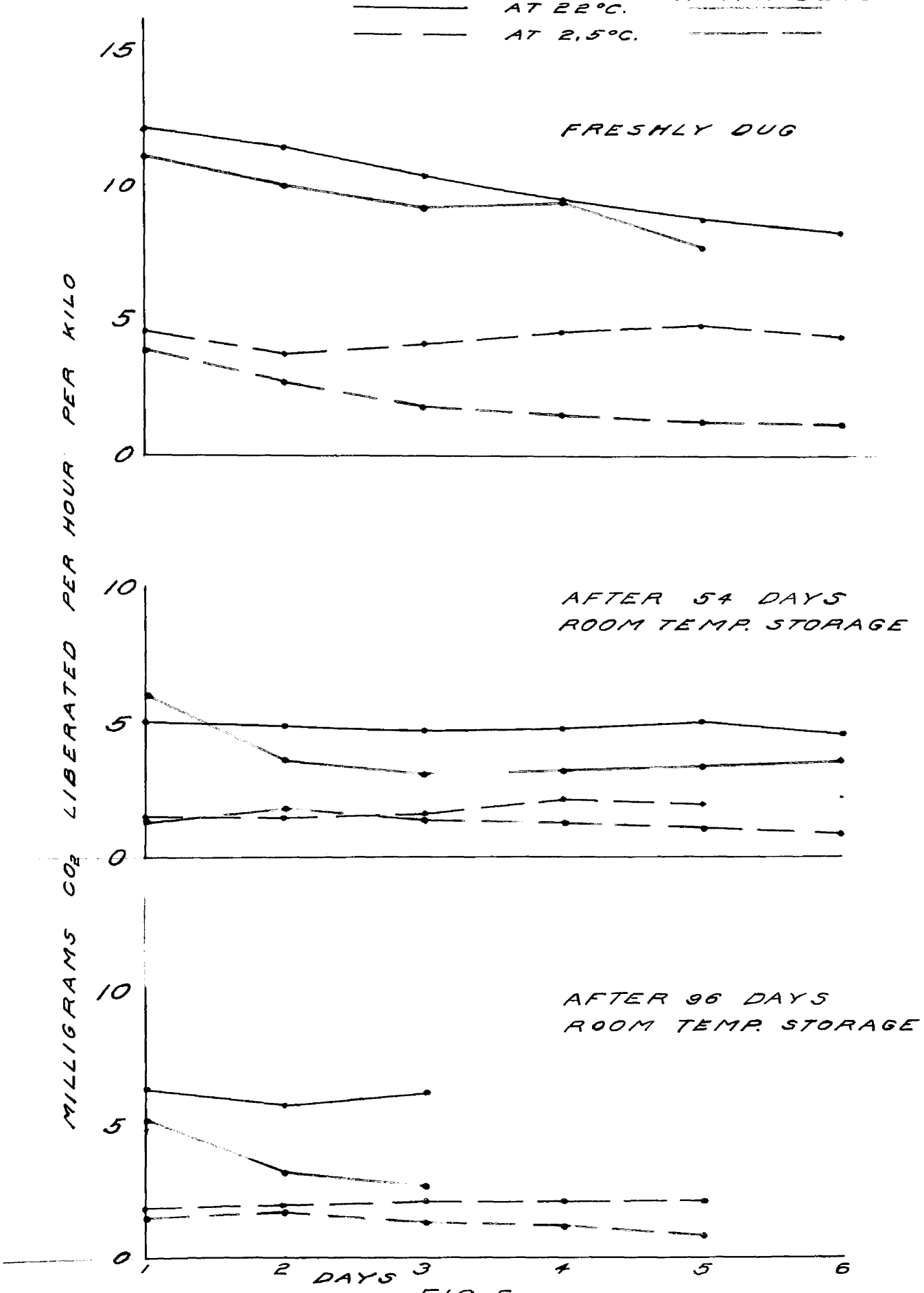

RESPIRATION OF
IRISH COBBLER POTATOES
AEROBIC
AT 22°C.
AT 2.5°C.
ANAEROBIC
FRESHLY DUG
AFTER 54 DAYS
ROOM TEMP. STORAGE
AFTER 96 DAYS
ROOM TEMP. STORAGE
MILLIGRAMS CO_2 LIBERATED PER HOUR PER KILO
DAYS
0
5
10
15
1
2
3
4
5
6

FIG. 5.

Experiment 3.-- Four samples of McCormick potatoes were used in this experiment. These had been harvested about two weeks, and had been stored at about 45° F. (7° C.) since digging. They were at room temperature for three days before the test was begun. The results are given in table 28.

Table 28. Respiration rates of McCormick potatoes after two weeks cellar storage after digging. Begun, November 20, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 22° C.			at 2±1.0° C		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	29.8	16.8	.56	7.1	5.7	.80
2nd. day	23.5	14.6	.62	5.3	4.1	.79
3rd. day	20.3	12.4	.61	5.8	3.0	.52
4th. day	16.7	aerobic 40.8	---	5.6	2.1	.37
5th. day	15.1	36.7	---	6.0	2.00	.33
6th. day	15.3	19.8	---	6.4	1.2	.19
7th. day	12.9	16.3	---	5.1	2.4	

Experiment 4.-- The purpose of this experiment was to determine the relative decrease in the I/N value of potatoes after storage at alternately high and low temperature. For this purpose, four samples of McCormick potatoes were used. The samples were divided into two lots; both samples in a lot received identical treatment in regard to temperature, but one sample was used to determine the aerobic respiration, the other the anaerobic. Each sample consisted of eight tubers and weighed about 900 grams.

The samples were selected about two weeks after the tubers were

harvested. One lot was stored in air seven weeks at 1.5 ± .5° C.; the two samples were then transferred to a temperature of 22 ± .5° C. and the aerobic and anaerobic respiration determined for six days. The anaerobic samples was continued for only four days to prevent serious injury due to anaerobiosis. Both samples were then stored in air at 22° C. for an additional four weeks, making a total of five weeks at 22° C. The samples were then transferred to the temperature of 1.5° C. and the aerobic and anaerobic respiration rates determined for six days. Both samples were then stored three weeks in air at 1.5° C., then transferred to a temperature of 22° C. and the respiration rates determined for five days; the anaerobic sample was run four days, then changed to air for the last day.

The other lot was run concurrently with the first, but kept at the opposite temperature throughout. It was first stored at 22° C., then transferred to 1.5° C. and the respiration determined. After a total of five weeks at 1.5° C., the samples were transferred to 22° C. and the respiration rates determined. After a total of four weeks at 22° C., the samples were again placed at 1.5° C. and the respiration measured.

The McCormick potatoes (tab. 29) which had been at 22° C. from January 8 to February 12 were sprouting and the I/N value was high. The same was true of the potatoes kept at 22° C. from February 12 to March 12. The tubers which had been kept at 1.5° C. from February 12 to March 12 had the sprouts which had begun to develop at the previous high temperature, but the sprouts were not actively growing and the initial I/N values are not large. The data for this experiment are presented in table 29.

Table 29. Respiration of McCormick potatoes after alternate periods of warm and cold storage

Time	Carbon dioxide liberated per hour per kilo					
Nov. 20-Jan. 8	Storage at 22° C.			Storage at 1.5° C.		
	Respiration at 1.5° C.			Respiration at 22° C.		
	aerobic mg.	anaerobic mg.	I/N	aerobic mg.	anaerobic mg.	I/N
Jan. 9	1.6	1.4	.84	32.0	23.1	.72
Jan. 10	2.2	1.9	.84	37.4	24.0	.64
11	2.3	1.8	.76	31.0	21.3	.69
12	2.2	1.4	.65	24.7	20.4 aerobic	.83
13	2.3	1.1	.48	17.5	40.1	---
14	2.8	.8	.31	14.5	43.2	---
Jan 14-Feb. 12	aerobic storage at 1.5° C			aerobic storage at 22° C.		
	Respiration at 22° C.			Respiration at 1.5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
Feb. 13	20.0	18.8	.94	1.7	2.5	1.44
14	26.0	21.1	.81	1.6	2.0	1.28
15	21.3	18.4	.86	1.9	1.9	.97
16	17.5	15.8	.90	2.1	1.6	.77
17	13.1	13.7	1.04	2.1	1.6	.74
18	11.9	aerobic 28.1	---	2.3	1.7	.75
Feb. 18-Mar. 12	aerobic storage at 22° C.			aerobic storage at 1.5° C.		
	Respiration at 1.5° C.			Respiration at 22° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
Mar. 13	2.0	3.0	1.52	13.9	13.6	.98
14	1.9	2.2	1.17	15.2	11.3	.75
15	2.0	1.8	.90	12.9	8.7	.68
16	1.6	1.3	.81	10.0	7.7	.77
17	2.2	1.2	.55	9.4	aerobic 13.3	---

While not the case in every test reported, there seems to be a tendency for the anaerobic respiration to decrease more rapidly than the aerobic when at low temperature. The Russet Burbank tubers (tab. 26) and the Irish Cobbler tubers after 96 days storage (tab. 27) do not show this effect clearly. The other Irish Cobbler samples, and the McCormick samples show very plainly the greater decrease in the respiration of anaerobic lots at low temperatures. The greater relative decrease in the anaerobic respiration results in lower I/N values for samples run at low temperature.

The theory that the decrease in anaerobic respiration is due to the toxic, or inhibitory action of the products of anaerobic respiration does not satisfactorily explain the greater decrease in the anaerobic respiration at low temperature because the products are not formed in such great quantity at the low temperatures.

Onions (*Allium cepa* L.)

The onions used in this experiment were of the yellow globe variety, and were bought at the local Sanitary Grocery, April 16, 1932.

Loose outer scales of the bulbs were removed, and four comparable samples of 17 bulbs each were selected. The bulbs were small in size; the samples weighed from 645 to 744 grams each.

Two samples were placed in the respiration chambers immediately, and the determination of the aerobic and anaerobic respiration was begun. The other samples were stored for 18 days at 2.2° C. (36° F.) before beginning the determination of the respiration.

Samples for moisture and sugar determinations were taken from smaller samples of similar bulbs.

The samples stored at 2.2° C. were allowed a preliminary period of three hours to come to the temperature of the respiration chambers; the record on the unstored samples was begun after a preliminary period of one hour.

The aerobic samples developed extensive roots and new leaf growth started. Only small areas of Penicillium appeared, insufficient to make a noticeable error in the respiration. The results are presented in table 30.

Table 30. Respiration rates of onions at 22° C.

Respiration period	Carbon dioxide liberated per hour per kilogram fresh weight					
	Bulbs not stored			After storage 18 days at 36°F(2.2°C)		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	47.8	33.1	.69	52.8	28.7	.54
2nd. day	42.0	23.4	.56	53.6	21.0	.39
3rd. day	39.3	20.0	.51	48.5	18.0	.37
4th. day	37.3	19.3 aerobic	.52	48.2	16.1	.33
5th. day	38.2	39.7	---	47.3	17.5	.37
6th. day	38.4	49.4	---	48.2	18.2	.38
7th. day	38.1	49.9	---	48.6	19.2 aerobic	.40
8th. day	35.0	51.4	---	46.4	40.0	---
9th. day	36.8	49.0	---	45.4	56.8	---
10th. day	36.4	51.3	---	45.1	57.1	---
11th. day	36.2	50.4	---	48.6	62.5	---
12th. day	36.5	49.6	---	44.8	58.0	---
13th. day	----	----	---	45.7	61.9	---

Table 31. Moisture and sugar analyses of onions before and after storage at 36° F. (2.2° C.)

Storage		Moisture	Reducing sugars as dextrose		Total sugars as invert	
Time	Temp.		fresh wt.	dry wt.	fresh wt.	dry wt.
		per cent	per cent	per cent	per cent	per cent
0	----	90.17	3.96	40.80	5.81	59.13
18 days	2.2° C. (36° F)	89.96	4.46	44.41	7.29	72.66

Discussion

Onions increase somewhat in sugar content during cold storage, though not so markedly as potatoes (tab. 25). The aerobic respiration was slightly stimulated by the cold storage, but this was not true of the anaerobic respiration. The I/N values for the two tests differ for this reason. The results of the first days' run indicates that the preliminary periods were not long enough to displace all the free oxygen in the respiration chambers and in the bulbs, because the anaerobic respiration rate is much lower for the second day than for the first. A less probable explanation for the high anaerobic respiration during the first day is that the nitrogen exerted a stimulus. The anaerobic respiration of the onions after cold storage began to increase after the fourth day; this increase may indicate some injury or breakdown of tissue.

All the samples produced both leaf and root growth by the end of the test periods but the anaerobic samples did not show growth until changed to air. A little blue mold (Penicillium) appeared under anaerobic conditions.

From this experiment, the conclusion seems warranted that a period in cold storage does not stimulate the anaerobic respiration of onions when removed to a higher temperature.

Tomatoes (Lycopersicon esculentum L.)

The tomato fruits used in the following experiments were grown by the Department of Horticulture, and made available for this work through the courtesy of Dr. Cordner and Mr. Frazier.

Experiment 1.-- This preliminary experiment was started September 28, 1932. Green fruits were picked at random from the plot used by Mr. Frazier in his studies on the cracking of fruits. The fruits were not all of the same variety, but they were all at about the same stage of ripeness. They were of the light green color which immediately precedes the pink stage. All of the fruits were small, being the last of the crop at the end of a rather dry season. Samples of fifteen fruits each were used for the respiration tests. Each sample contained approximately the same number of fruits of each different size. The weights of the samples varied from 1278 to 1395 grams.

The fruits were picked early in the morning and placed in the respiration chambers by 9:00 A. M. Carbon-dioxide-free air, or nitrogen was passed rapidly through the chambers until 10:20 A. M. when the record was begun. The respiration temperatures were 22° C. and 3 to 4.5° C. The results are presented in table 32.

Table 32. Respiration of green tomato fruits.

Respiration period	Carbon dioxide liberated per hour per kilogram fresh weight					
	at 22°C.			at 3 to 4.5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	47.2	48.9	1.04	13.6	17.9	1.31
2nd. day	44.2	38.0	.86	5.7	10.2	1.78
3rd. day	50.4	28.8	.57	7.4	9.0	1.21
4th. day	61.0	25.4	.42	8.5	9.2	1.08
5th. day	----	----	----	10.4	8.9	.86
6th. day	----	----	----	11.4	9.4	.83
7th. day	----	----	----	11.8	10.0	.84
				Changed to 22° C.		
8th. day	----	----	----	55.5	43.7	.79
9th. day	----	----	----	46.3	29.4	.64
10th. day	----	----	----	51.0	22.6	.44
11th. day	----	----	----	49.2	20.3	.41

At the end of four days, three of the fruits of the aerobic lot in table 32 showed a slight growth of Fusarium on the surface. Ten fruits had developed a pink or red color. In the anaerobic lot two fruits showed a little exudate, perhaps due to bacterial action; four had developed a little pink color; all had an abnormal odor.

At the end of seven days at low temperature, only the aerobic lot showed any change, a very slight ripening. After removal to 22° C., eight fruits of the aerobic lot turned pink. Four fruits in the anaerobic lot had turned faintly pink, but the color was not normal; the fruits were soft, and their odor abnormal.

Experiment 2.-- In the summer of 1933, better fruits were available and experiments were carried out on fruits at different stages of ripeness.

The fruits were of the Marglobe variety. They were grown on weed infested soil and the fruits were small for the variety. Each respiration sample consisted of eight fruits and weighed about 950 grams. In all cases, the fruits were picked about noon, cleaned by wiping gently with a moist cloth, sorted, weighed and placed in the respiration chambers about 3:00 P. M. The preliminary period lasted about 18 hours before the record was begun.

Fruits were studied at three stages of ripeness, approximating the stages shown in color by Sando (1920). The green fruits were usually light green in color, just before the appearance of pink; pink fruits were colored pink over a part of the surface, seldom more than half; red ripe fruits were completely red, but did not show signs of softening. The ages of the fruits were not known.

The warm temperature was 28° C. for these experiments; the cold chambers were not so accurately controlled, but varied between 5 and 6° C. The results are given in tables 33, 34, and 35 and shown in figure 6.

FIG. 6.

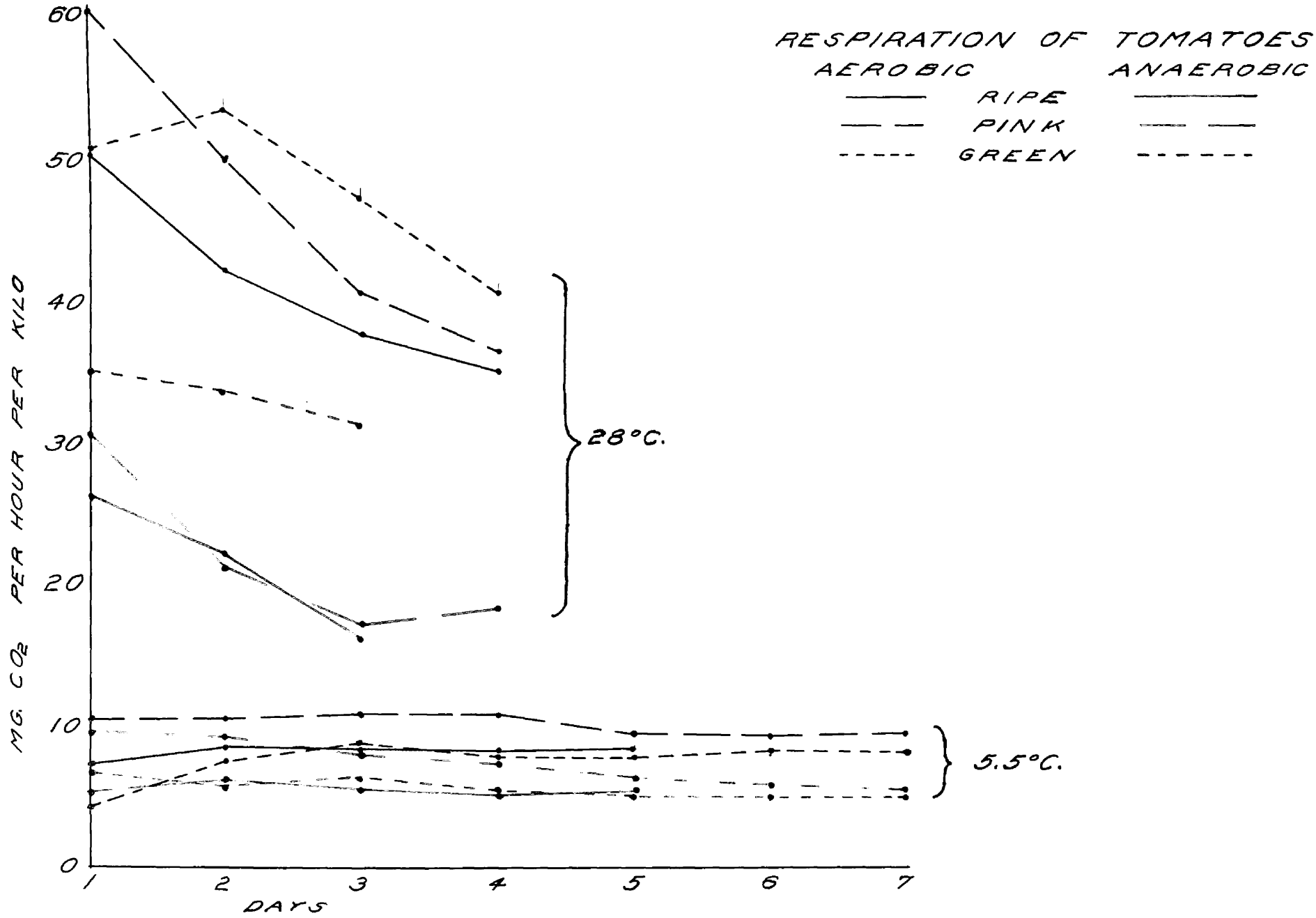

RESPIRATION OF TOMATOES
AEROBIC
ANAEROBIC
RIPE
PINK
GREEN
MG. CO_2 PER HOUR PER KILO
60
50
40
30
20
10
0
1
2
3
4
5
6
7
DAYS
28°C.
5.5°C.

Table 33. Respiration of green Marglobe tomato fruits. August 25 - September 2, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 28 ± 0.2° C.			at 5.5 ± .5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	50.6	35.1	.69	4.1	6.8	1.68
2nd. day	53.4	33.8	.63	7.5	6.0	.80
3rd. day	47.3	31.3	.66	8.7	6.5	.75
4th. day	40.8	----	---	7.9	5.4	.68
5th. day	----	----	---	8.0	5.2	.65
6th. day	----	----	---	8.4	5.1	.61
7th. day	----	----	---	8.1	5.1	.62

Table 34. Respiration of Marglobe tomato fruits in the pink stage. August 17 - 25, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 28 ± .2° C.			at 5.5 ± .5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	60.2	30.8	.51	10.3	9.9	.97
2nd. day	50.0	21.5	.43	10.6	9.2	.87
3rd. day	40.9	17.4	.43	11.0	8.1	.73
4th. day	36.7	18.6	.51	11.0	7.6	.69
5th. day	34.0	----	---	9.5	6.6	.70
6th. day	----	----	---	9.4	6.2	.66
7th. day	----	----	---	9.7	5.6	.58

Table 35. Respiration of red ripe Marglobe tomato fruits. Aug. 11-17, 1931.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 28 ± 0.2° C.			at 5.5 ± .5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	50.2	26.6	.53	7.4	5.4	.73
2nd. day	42.0	22.3	.53	8.6	6.2	.72
3rd. day	37.8	16.3	.43	8.5	5.7	.67
4th. day	35.3	----	---	8.2	5.1	.62
5th. day	----	----	---	8.5	5.3	.62

Discussion

The fruits were kept under anaerobic conditions at 28° C. until breakdown of tissue occurred, which came with surprising suddenness. Breaks in the tables are due to the discard of samples showing injury. The results of the first three days of 28° C. are considered reliable; in the cold the fruits held up better, and the data presented is thought to give a true estimate of the respiration throughout the entire period at the low temperature.

None of the Marglobe fruits developed any red pigment in the absence of oxygen.

The aerobic respiration rates of the fruits used in this experiments are about the same as Gustafson reported (1929). The fruits in the pink stage had the highest aerobic respiration. The respiration decreased sharply as the fruits ripened further.

The green fruits had the highest anaerobic respiration at the warm temperature, both in actual quantity of carbon dioxide liberated, and in proportion to the aerobic respiration.

The fruits in the pink stage produced more carbon dioxide at low temperature than fruits at other stages, both under aerobic and anaerobic conditions.

The I/N values were about the same for all stages, but were consistently higher at the low temperature than at the high temperature. This may be due to the toxic, or inhibitory, effect of the products of the anaerobic respiration which accumulate more rapidly at the higher temperature, or it may be due to the low energy release of the anaerobic processes.

Carrots (Daucus carota L.)

The experiments with carrots and parsnips were designed to extend the studies of Smith (1929) who determined the aerobic respiration of a number of vegetables after a period of cold storage.

Material.-- The carrots were purchased as needed from the local groceries. They were always in good condition with the tops still green, but were by no means freshly harvested. The tops (leaves) were cut off about one-fourth inch above the crown before using. Since the roots varied in size and shape, samples were sorted carefully to eliminate differences as nearly as possible.

Experiment 1.-- The samples for this experiment consisted of 10 roots each, and weighed from 773.5 to 785.0 grams each. Cut surfaces were dipped in 1:1000 mercuric chloride solution for partial sterilization. The preliminary period was of 16 hours duration. The results are presented in table 36.

Table 36.--Respiration of carrots. Begun May 1, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 22 - 25° C.			at 3 - 4° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	53.2	60.2	1.13	14.6	15.3	1.05
2nd. day	50.5	60.7	1.20	13.8	13.3	.97
3rd. day	54.9*	60.4	(1.11)	13.4	13.6	1.01
4th. day	----	----	----	13.7	13.1	.96
5th. day	----	----	----	12.8	12.2	.95
6th. day	----	----	----	11.8	12.0 aerobic	1.01
7th. day	----	----	----	10.4	14.0	----

* Mold starting.

The carrots in this experiment showed very high anaerobic respiration and since the temperature of the warm bath fluctuated widely, due to high diurnal temperature, the experiment was repeated, with the warm bath set at 28° C.

Experiment 2.-- The respiration samples of the second experiment consisted of twelve roots each and weighed from 569.5 to 581.5 grams. A similar collateral lot was analysed for sugars and moistures, following the procedure already described. At the end of six days the sugar and moisture samples were taken from the respiration samples run at 28° C. The respiration data is presented in table 37; the sugar and moisture percentages in table 38.

Table 37. Respiration of carrots. Begun May 9, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 28 ± .2° C.			at 3 - 4° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	68.2	105.5	1.54	14.1	12.5	.89
2nd. day	60.6	105.1	1.74	12.2	11.0	.90
3rd. day	52.6	100.8	1.92	12.3	10.0	.82
4th. day	51.3	102.2	1.99	12.4	11.1	.90
5th. day	46.4	105.9*	(2.29)	13.3	11.6	.87
6th. day	40.0	114.3*	----	11.8	11.9	1.00

* Noticeable bacterial growth, but not extensive.

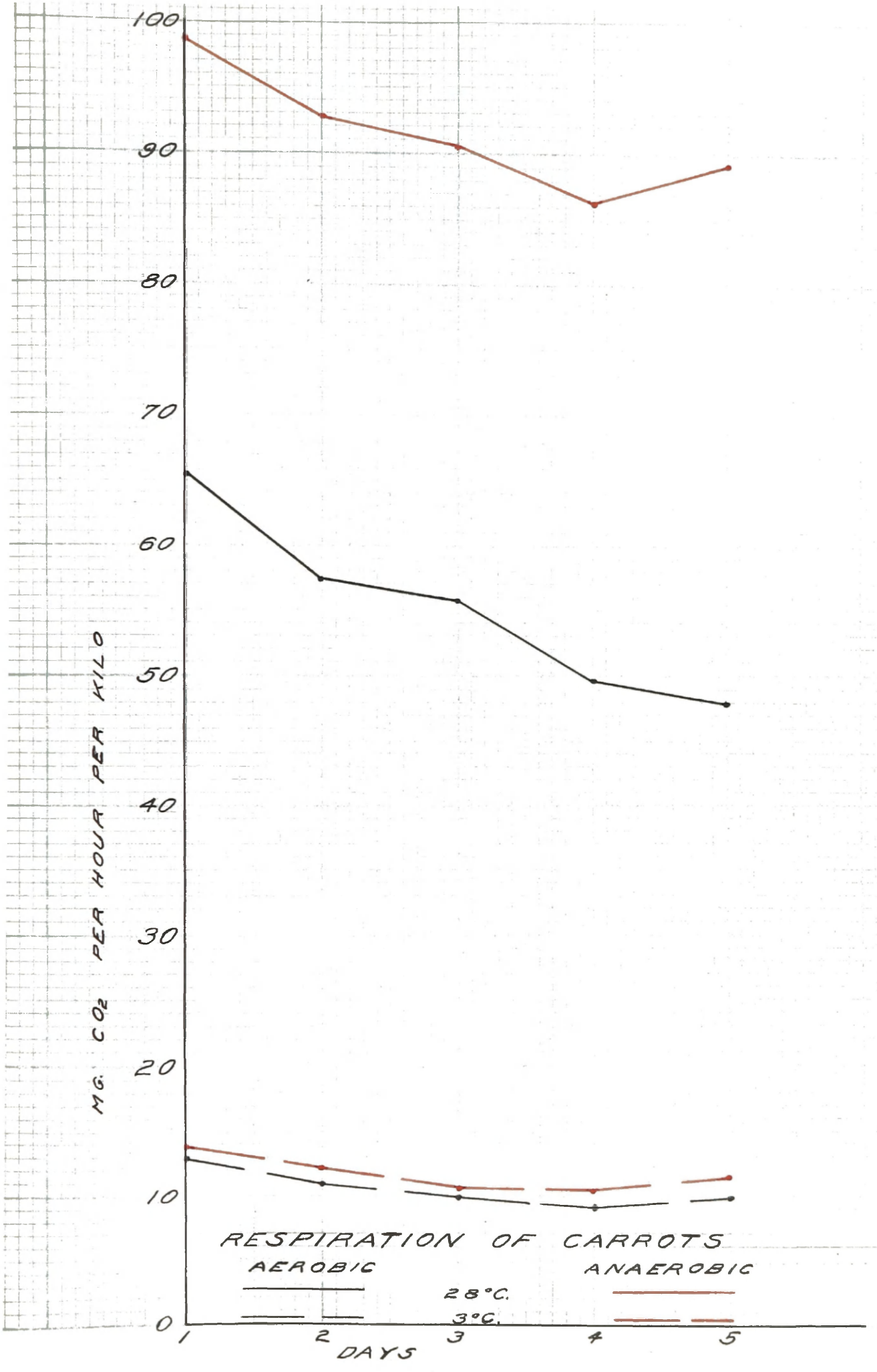

100
90
80
70
60
50
40
30
20
10
0
MG. CO_2 PER HOUR PER KILO
1
2
3
4
5
DAYS
RESPIRATION OF CARROTS
AEROBIC
ANAEROBIC
28°C.
3°C.

Table 38. Sugar and moisture content of carrots, before and after respiration.

Storage			Moisture	Reducing sugars as dextrose		Total sugars as invert	
Period	Temp.	Atm.		wet wt.	dry wt.	wet wt.	dry wt.
			per cent	per cent	per cent	per cent.	per cent.
0	----	----	87.11	3.56	27.65	7.29	56.58
6 days	28° C.	air	87.95	3.10	25.77	6.50	53.98
		N_2	89.71	3.07	29.79	4.23	41.08

Experiment 3.-- A third series of samples was run later to check previous work. The samples consisted of eight roots each and weighed from 486 - 496 grams each. All were dipped for 3 minutes in 50 per cent alcohol, then rinsed, before placed in chambers. A previous test had shown that this treatment did not noticeably affect the respiration rate. Sugar and moisture samples were taken from a collateral sample at the beginning of the experiment, and from both the aerobic and anaerobic samples at the end of the respiration test, The test was run 5 days and 18 hours; the warm temperature was 28° C. and the cold 3 ± .5° C. The preliminary period lasted 18 hours. The results are presented in table 39, and shown in figure 7. The sugar and moisture percentages are given in table 40.

Table. 39. Respiration of carrots at high and low temperatures. Begun, September 19, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 28 ± .3° C.			at 3 ± .5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	65.6	98.7	1.50	12.8	13.7	1.07
2nd. day	57.4	92.7	1.62	11.0	12.3	1.12
3rd. day	55.7	90.6	1.63	9.9	10.8	1.09
4th. day	49.7	86.4	1.74	9.1	10.6	1.17
5th. day	48.0	89.3	1.86	9.8	11.4	1.15

There was no mold growth on any sample at the conclusion of this test. The aerobic sample at 28o C. showed a little growth of leaves at the crown but there was no growth on the anaerobic sample.

Table 40. Sugar and moisture content of carrots used in respiration test, September 19, 1933.

Storage			Moisture	Reducing sugars as dextrose		Total sugars as invert	
Period	Temp.	Atm.		wet wt.	dry wt.	wet wt.	dry wt.
			per cent	per cent	per cent	per cent	per cent
0	----	----	88.83	2.27	20.37	6.11	54.66
5 days	28° C.	air	88.93	2.25	20.34	6.08	54.94
		N_2	91.41	1.59	18.53	3.46	40.27

Experiment 4.-- The purpose of this experiment was to test the effect of ethyl bromide treatment on the respiration of carrots.

All four samples were placed in a 12 liter desiccator, and 5 cc. of ethyl bromide allowed to evaporate from cotton at the top of the container. After two hours exposure, the samples were removed to the respiration chambers as quickly as possible.

The samples consisted of ten carrots each and weighed from 563 to 568 grams. The preliminary period was 16 hours. There were no untreated controls but comparison should be made with untreated lots of the test run May 9, 1933 (tab. 37). For results after ethyl bromide treatment, see table 41.

Table 41. Respiration rates of carrots after ethyl bromide treatment.

May 17, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 28 ± .3° C.			at 3 ± 1.0° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
16 hrs. Prelim.	97.4	(112.5)	(1.16)	(29.8)	(29.8)	(1.00)
1st. day	74.3	111.3	1.49	15.9	18.3	1.15
2nd. day	61.3	100.9	1.65	14.2	17.4	1.22
3rd. day	55.5	100.1	1.80	14.0	17.7	1.26
4th. day	54.4	(105.2)	(1.93)	14.4	17.0	1.18
5th. day	(53.0)	(106.2)	(2.00)	14.3	16.6	1.16
6th. day	(55.6)	(107.1)	(1.92)	12.4	14.4	1.16

Values in parentheses are probably unreliable. At the beginning, incomplete anaerobiosis perhaps results in values too high. At the low temperature, the respiration is high during the preliminary period, because the carrots were warm at the start and required some time to reach the low temperature. Values during the fourth to sixth days are in error due to mold and bacterial growth.

Experiment 5.-- A series of carrots was used to test the relative inertness of hydrogen and nitrogen gases in study of anaerobiosis. Three samples of eight roots each were used; the weights of the samples varied from 373.5 to 381.5 grams each. All roots were dipped in 50 per cent alcohol for 3 minutes, rinsed in distilled water for 2 minutes, and dried before placing in the respiration chambers. The preliminary period lasted 15 hours. When a change was made from one gas to another, the second gas was introduced at the beginning of the purification train about 20 minutes before a day's

run ended; even so, the second gas would not displace the previous one until several hours had elapsed. The results are given in table 42.

Table 42. Respiration of carrots in air, nitrogen, and hydrogen at 27.8 ± .2° C

October 19 - 26, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					I/N
	aerobic	Anaerobic				
		medium	sample 1	sample 2	average	
	mg.		mg.	mg.	mg.	
1st. day	67.5	Nitrogen	118.6	113.7	116.2	1.72
2nd. day	60.7	Hydrogen	109.6	113.6	111.6	1.84
3rd. day	60.9	Hydrogen	104.0	107.8	105.9	1.74
4th. day	55.8	Nitrogen	93.6	101.4	97.5	1.82
5th. day	55.4	Nitrogen	91.4	100.4	95.9	1.73
6th. day	56.8	Hydrogen	96.2	110.6	102.4	1.80
7th. day	54.3	Nitrogen	90.4	97.8	94.1	1.73

Discussion

The experiments reported show a consistent high anaerobic respiration for carrots. The I/N values are highest at the warm temperature; usually the I/N value of the first days run at the warm temperature was about 1.50 and it usually increased as time went on. In the cold, the I/N value was about unity and remained fairly constant.

The high anaerobic respiration rate was certainly not due to infection by bacteria or fungi. There was no visible mold at any time, nor any sign of bacterial infection in the series run September 19, 1933 (tab. 39). The odor and taste of the roots were nearly, or quite normal after a week of anaerobiosis, and the appearance of the roots was normal. The tissue, after grinding on a Nixtamal mill, seemed more juicy, and the results

(tables 38 and 40) show a higher moisture content. Whether this increase in moisture is actual, or apparent, is unknown, because volatile end products of anaerobic respiration, such as alcohol, would cause an apparent increase in the moisture content.

The anaerobic respiration of carrots was about the same in either hydrogen or nitrogen (tab. 42). From the results reported, hydrogen seems to cause a slight increase in the amount of carbon dioxide produced when substituted for nitrogen. On the other hand, a decrease occurs when nitrogen follows hydrogen. The I/N values are not affected significantly by the slight changes in respiration resulting from the alternation of gases used.

It is difficult to estimate the effect that ethyl bromide treatment has on carrots (tab. 41) because untreated controls were not run at the same time. Comparison is best made with untreated samples given in table 37. The slight stimulus possibly due to the ethyl bromide is of short duration, about 1 day, and seems more pronounced in the aerobic samples, and at the warm temperature.

The total sugar content of carrots decreases markedly during anaerobic respiration (tables 38 and 40). The amounts of reducing sugars did not change appreciably in one analysis but decreased more significantly in the experiment 3. The greater decrease in the sugar content of the anaerobic samples indicates that sugar is probably the chief food of the carrot, and is the substance broken down during respiration. Much more material is used in anaerobic splitting to form a given amount of carbon dioxide than in aerobic processes. This may account for the greater decrease in the sugar content of the anaerobic lots.

Gustafson (1932) found that several different species of cacti give off about as much, or slightly more, carbon dioxide by anaerobic processes

as by aerobic oxidation. A comparison of the food reserves in the two materials would be interesting because of their similar respiratory behavior.

Richards (1896) found that carrots had a high anaerobic respiration, about equal to aerobic, but his experiments did not run over periods as long as used in the present work.

Parsnips (Pastinaca sativa L.)

This experiment was planned to extend the experiments performed by Smith (1929) on the respiration of parsnips after cold storage, by including the determination of the anaerobic respiration.

The parsnips were purchased from a local trucker, November 28, 1933, being dug expressly for this experiment. The roots were dug in the morning, washed just before noon, and the samples sorted out just after noon. The samples consisted of seven roots each; those used immediately for the determination of respiration weighed about 640 grams each and the samples placed in cold storage weighed about 540 grams each.

Four samples were placed in the respiration chambers immediately after sorting to determine the respiration of freshly dug parsnips. The remaining samples were stored in moist loam soil at 2.2° C. (36° F.) for 30 days. The method of storage is described by Smith (1929). These samples were then removed and respiration tests made. The preliminary periods lasted 20.25 hours in each instance; the respiration tests were made at 22 ± .3° C. and at 1.5 ± .5° C.

Samples for sugar and moisture determinations were taken from collateral samples at the beginning of the respiration tests at 22° C.; samples for the determination of sugars and moisture were taken from the respiration samples.

The results of the respiration tests are presented in tables 43 and 44 and shown in figure 8. The percentages of moisture and sugars are given in table 45.

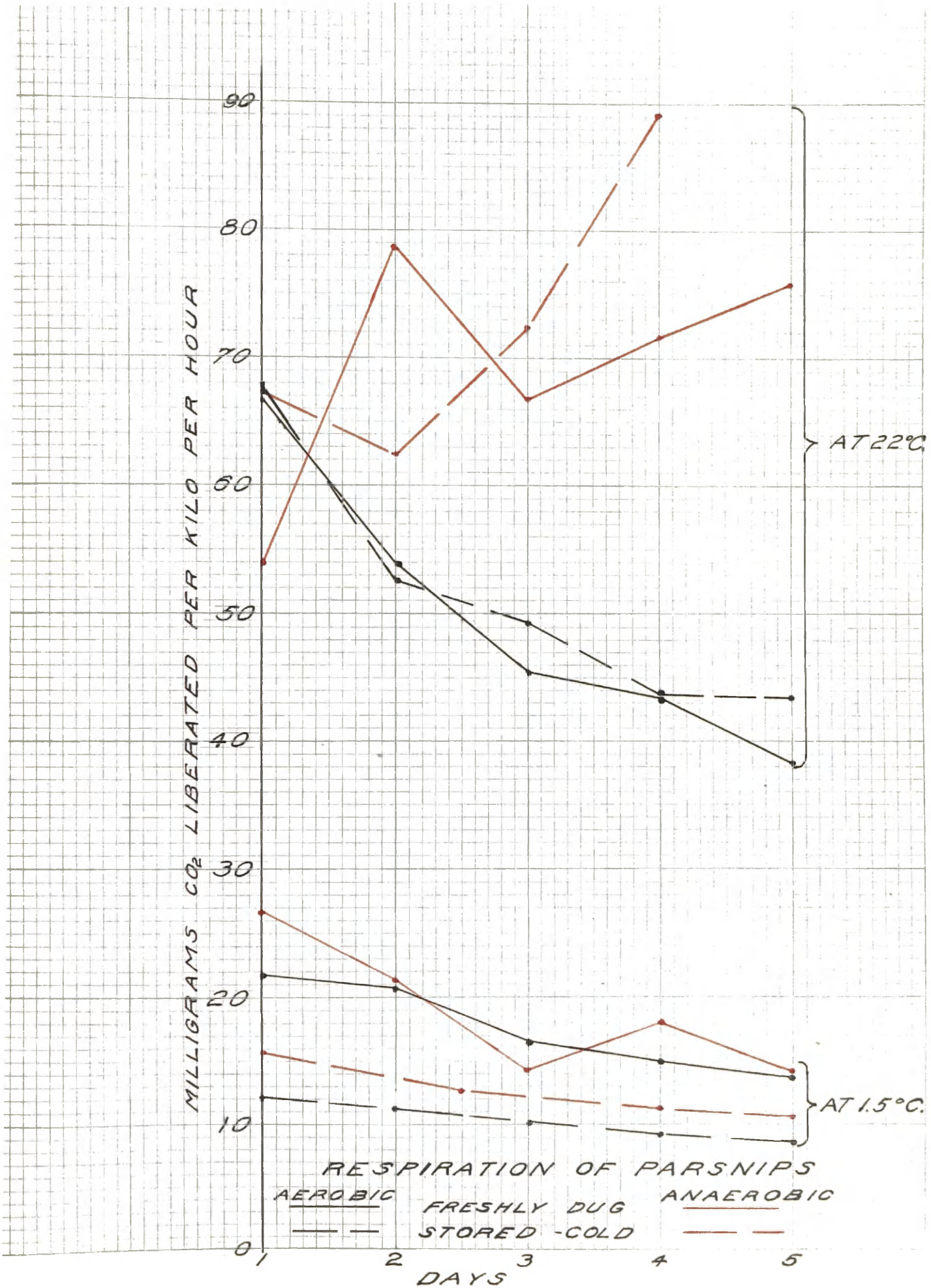

90
80
70
60
50
40
30
20
10
0
MILLIGRAMS CO_2 LIBERATED PER KILO PER HOUR
AT 22°C.
AT 1.5°C.
RESPIRATION OF PARSNIPS
AEROBIC
FRESHLY DUG
ANAEROBIC
STORED -COLD
1
2
3
4
5
DAYS

FIG. 8.

Table 43. Respiration rates of freshly harvested parsnips. Begun, Nov. 28, 1933

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 22 ± .3° C.			at 1.5 ± .5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	66.7	53.9*	.81	21.8	26.8	1.23
2nd. day	53.9	78.8	1.46	20.8	21.4	1.03
3rd. day	45.5	66.7	1.47	16.6	14.2	.86
4th. day	43.4	71.6	1.65	15.0	18.0	1.20
5th. day	38.2	(75.9)	1.99	13.7	14.1	1.03

* The low value for the first day's anaerobic record at 22° C. was probably due to poor adjustment of the gas flow because the value for the second day is higher than expected.

Nitrogen was used during the preliminary period and for 10 hours the first day. It was then necessary to change to hydrogen for the remainder of the test.

Table 44. Respiration rates of parsnips after 30 days storage at 1.0 — 2.5° C. Begun, December 28, 1933.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 22 ± .3° C.			at 1.5 ± .5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day.	65.8	67.3	1.02	12.1	15.7	1.30
2nd. day	52.6	62.4	1.19	10.7*	12.7*	1.18*
3rd. day	49.4	72.3	1.46			
4th. day	43.6	(89.1)	(2.04)	9.2	11.3	1.23
5th. day	43.4	(118.5)	(2.73)	8.7	10.6	1.21

* Average of 2 days.

Table 45. Sugar and moisture content of parsnips.

Treatment of samples			Moisture	Reducing sugars as dextrose		Total sugars as invert sugar	
Storage at 1.5° C.	Respiration at 22° C.			Wet wt.	dry wt.	wet wt.	dry wt.
days	period	atm.					
	hours		per cent	per cent	per cent	per cent	per cent
0	0	---	81.48	.27	1.44	6.14	33.16
	140	air	81.39	.77	4.11	5.79	31.11
	140	N_2	82.45	.31	1.76	5.25	29.92
30	0		80.52	1.04	5.36	9.26	47.52
	140	air	80.74	.84	4.34	8.74	45.35
	140	N_2	82.75	.88	5.09	7.40	42.90

Discussion

The parsnips used did not show any significant increase in respiration after storage at low temperature. This may be due to the fact that the roots had been subjected to some rather cold weather before digging. The aerobic respiration rates of both the freshly dug roots and the stored ones decreased during the course of the experiment. Smith (1929) has already reported similar data. At the warm temperature, the anaerobic respiration of parsnips did not decrease during the experiments. Since the aerobic rate decreased while the anaerobic rate remained relatively constant, the I/N values increased.

The anaerobic respiration showed an apparent increase after a few days. That this was due to infection with micro-organisms is doubted because there was no mold, or visible sign of bacteria, even when the roots were cut open. The slight top growth of the aerobic lots did not seem to appreciably affect

the rate of respiration. It was unfortunate that the use of hydrogen was compulsory in the first experiment, but, except for the one possible error already mentioned, the figures are considered reliable, and not materially affected by the change from nitrogen to hydrogen (See also table 42).

The respiration of freshly dug parsnips is much higher at the cold temperature than that of roots after a period of cold storage, despite the fact that the sugar content of the stored roots was markedly higher than that of the freshly dug roots.

The weights of the soil stored lots remained almost unchanged for the 30 day storage period. The sugar content (tab. 45), both of reducing and total sugare increased significantly during storage. There was always a greater decrease in the total sugar content during anaerobic than during aerobic respiration. The figures are so conflicting that any conclusion concerning the change in reducing sugars is unwarranted.

From the data reported it may be concluded that parsnips have a high anaerobic respiration in comparison to the aerobic. In this respect they resemble carrots.

The sugar content of parsnips increases during cold storage. This increase in sugar content is not correlated with an increase in respiration when the roots are removed to a higher temperature. This observation supports Smith's (1929) work. Parsnips differ from potatoes in this respect.

Turnips (*Brassica rapa* L.)

The turnips used in this experiment were purchased in a local grocery, March 19, 1934. They had been shipped in from the south, but the tops were still fairly fresh.

After removing the tops, four samples of five roots each were selected. Two samples were placed in respiration chambers immediately, and the respiration determined.

The remaining two samples were stored in open containers at 2.2° C. (36° F.) for two days. They lost weight rapidly, so they were placed in moist chambers with loosely fitting covers, and a piece of moist towel paper placed over the roots. After taking this precaution, the weights remained constant. The storage period lasted 17 days.

The respiration was determined at 22 ± .5° C. The preliminary periods were about 5.5 hours. The results are presented in table 46.

Table 46. Respiration of turnips at 22±0.5° C.

Respiration period	Carbon dioxide liberated per hour per kilo					
	Fresh roots			After 17 days storage at 36° F.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	69.8	47.5	.68	90.4	50.7	.56
2nd. day	66.2	40.4	.61	86.2	39.2	.45
3rd. day	58.5	41.7	.71	74.4	36.5	.49
4th. day	----	----	---	67.3	36.9	.55

Discussion

The turnips of both aerobic samples developed some new root and leaf growth. There was no leaf or root growth in the aerobic samples. A slimy growth of bacteria developed around the crowns of the turnips in the anaerobic samples, and a foul odor was present after the second day. Only the data for the first two days are considered reliable. Cold storage of turnips results in an increase in the aerobic respiration at subsequent high temperature, but the anaerobic respiration seems quite unaffected by such treatment.

Green Sweet Corn (Zea mays L.)

The following experiments were designed to study both the aerobic and anaerobic respiration of green sweet corn, and the concurrent changes in the carbohydrate reserve.

Experiment 1.-- The first experiments were carried out with corn of the Hopeland variety. This was supplied by the Agronomy Department of the Maryland Agricultural Experiment Station.

The ears were picked early in the morning and brought to the laboratory. They were then shucked, and the silks removed by brushing lightly. The butts were cut off smoothly, and grains injured by ear worms were cut out. The cut surfaces were swabbed with 50 per cent alcohol to reduce possible infection. The preliminary periods lasted two hours. In this interval, collateral lots were sampled for sugar and moisture analysis. For these analytical samples, three rows of grains were cut from each ear used, ground, mixed, and the samples weighed into flasks.

Two runs were made on corn in the milk stage. The ears were 19 days old from the time silks had first appeared. One run was made on corn in the early dough stage, the ears of which were 26 days old from the time of silking. The writer is indebted to Mr. Donald Goss of the Agronomy Department for the data relative to the age of the corn used. All the ears of a given age were not in the same physiological age, and ears were selected which seemed average for the stage used. The 'thumb nail' test was used in this final separation. The results of the respiration tests are given in tables 47 and 48; the changes in sugars and moistures are presented in table 49. The data are also shown in figure 9.

Table 47. Respiration at 30° C. of Hopeland sweet corn in the milk stage.

Respiration period	Carbon dioxide liberated per hour per kilo					
	Run August 15-22, 1932			Run August 23-30, 1933		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	493.2	388.2	.79	471.1	368.4	.78
2nd. day	418.1	236.4	.57	401.0	275.6	.69
3rd. day	370.4	186.2	.50	333.0	203.3	.61
4th. day	316.6	147.6	.47	301.7	160.7	.53
5th. day	301.0	118.4	.39	264.8	141.8	.54
6th. day	248.2	89.9	.36	222.4	112.5	.51
7th. day	202.9	70.4	.35	192.9	89.3	.46

Table 48. Respiration at 30° C. of Hopeland sweet corn in the dough stage.

Begun, August 31, 1932

Respiration period	Carbon dioxide liberated per hour per kilo		
	aerobic	anaerobic	I/N
	mg.	mg.	mg.
1st. day	398.9	271.7	.68
2nd. day	347.6	179.4	.52
3rd. day	298.5	141.2	.47
4th. day	258.6	139.5	.54
5th. day	221.5	131.9	.60
6th. day	188.4	119.3	.63

RESPIRATION OF HOPELAND SWEET CORN
AEROBIC
ANAEROBIC
MILK STAGE
DOUGH STAGE
SUGARS
REDUCING
INVERT
MG. CO2 PER HOUR PER KILO
100
200
300
400
500
0
1
2
3
4
5
6
7
DAYS
SUGARS - PER CENT DRY WT.
0
5
10
15
20
25
MILK
DOUGH
SUGARS - AFTER RESPIRATION
PER CENT DRY WT.
0
5
10
MILK
DOUGH

FIG. 9.

Table 49. Sugar and moisture content of Hopeland Sweet Corn.

Stage of ripeness when harvested	Storage at 30° C.		Moisture	Reducing sugars as dextrose		Total sugars as invert sugar	
	period	atm.		wet wt.	dry wt.	wet wt.	dry wt.
	days		per cent	per cent	per cent	per cent	per cent
Milk	0	---	77.85	.99	4.47	4.20	18.97
	7	air	74.95	.60	2.38	1.07	4.26
	7	N_2	81.11	2.36	12.49	2.35	12.44
Milk	0	---	80.62	1.21	6.25	4.94	25.47
	7	air	75.45	.44	1.80	.83	3.37
	7	N_2	78.69	1.77	8.32	1.96	9.17
Early dough	0	---	66.11	.94	2.78	3.49	10.29
	6	air	65.55	.50	1.46	1.14	3.31
	6	N_2	73.39	1.78	6.68	2.19	8.21

Experiment 2.-- Another experiment was run on corn in late milk stage purchased from a local market gardener, September 13, 1932. It was a white variety, called by the grower, Trucker's Corn.

Each sample consisted of four ears. Much damage had been caused by earworms, and the damaged kernels were all removed. The preliminary period lasted 3 hours. During this time a collateral sample was used for sugar, starch, and moisture samples.

Two respiration samples were run seven days at 30° C. The other two were run seven days at 3 1.0° C, then changed to 30° C. for six days. In changing temperature, the anaerobic lot was in air for some time because the samples were placed in different respiration chambers. A preliminary

period of 2 hours was run before starting the record again. The respiration results of this experiment are given in table 50; the changes in carbohydrates are expressed in table 51.

Table 50. Respiration rates of sweet corn in the milk stage, Trucker's variety

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 30° C.			at 3 ± 1.0° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	453.1	346.9	.77	75.4	59.7	.79
2nd. day	(222.9)*	190.5	(.85)	67.9	43.1	.63
3rd. day	321.3	148.7	.46	57.4	41.9	.73
4th. day	318.3	119.8	.38	61.8	41.8	.68
5th. day	291.0	101.4	.35	53.0	42.4	.80
6th. day	239.4	81.7	.34	49.9	39.3	.79
7th. day	217.6	71.3	.33	52.7	39.2	.74
				Changed to 30° C.		
8th. day	----	----	---	373.8	253.9	.68
9th. day	----	----	---	315.9	185.0	.59
10th. day	----	----	---	286.3	127.8	.45
11th. day	----	----	---	261.2	97.3	.37
12th. day	----	----	---	228.7	75.2	.33
13th. day	----	----	---	205.4	61.5	.30

* Air flow stopped in this chamber from 6:00 P. M. until 8:00 A. M. through faulty manipulation. Little carbon dioxide was lost, but its production greatly decreased, probably due to consumption of oxygen in container. Rate for first 5 hours of the second day was 383.6 mgs. per kilo per hour.

Table 51. Changes in carbohydrate and moisture content of Truckers' green sweet corn. Begun September 13, 1932.

Storage period at 3° C.	Storage period at 30° C.	Atm.	Moisture	Reducing sugars as dextrose		Total sugars as invert		Soluble polysaccharides (Dextrins)		Starch	
				wet wt.	dry wt.	wet wt.	dry wt.	wet wt.	dry wt.	wet wt.	dry wt.
			per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
0	0	----	75.61	.95	3.90	4.37	17.91	8.41	34.48	2.84	11.63
	7 days	air	76.97	.49	2.11	.95	4.13	5.45	23.65	3.98*	17.29*
	7 days	N_2	79.00	2.40	11.42	2.63	12.52	4.21	20.05	2.68*	12.76*
7 days	6 days	air	78.68	0.51	2.40	.93	4.38	4.76	22.31	3.46	16.22
		N_2	80.04	2.39	11.95	2.65	13.26	3.42	17.13	2.73	13.69

* Results of single analysis, all others values the averages of duplicate determinations.

Experiment 3.-- The last experiment with sweet corn was on Stowell's Evergreen variety, grown by the Department of Horticulture. The corn was picked in the morning, September 29, 1933, shucked, silked, and sorted into lots of four representative ears in each. Grains injured by earworm were cut out, and all cuts swabbed with 50% alcohol. The preliminary period lasted two hours, during which time samples were taken from a collateral sample, for moisture, sugar, and starch analyses. Samples for analysis were taken from the respiration samples at the end of the test at the high temperature.

The respiration was studied at 28° C. and at 4° C.

The corn, as determined by the 'thumb nail' test, was in the milk stage.

The results are presented in tables 52 and 53 and shown graphically in figure 10.

Table 52. Respiration rates of Stowell's evergreen sweet corn.

Respiration period	Carbon dioxide liberated per hour per kilo					
	at 27.8 ± .2° C.			at 4.5 ± 0.5° C.		
	aerobic	anaerobic	I/N	aerobic	anaerobic	I/N
	mg.	mg.		mg.	mg.	
1st. day	396.5	283.8	0.72	89.6	63.7	0.71
2nd. day	326.9	169.8	.52	68.3	37.8	.55
3rd. day	297.8	115.0	.39	67.3	38.5	.57
4th. day	271.6	93.8	.35	53.3	33.6	.63
5th. day	256.7	76.7	.30	49.5	29.6	.60
6th. day	229.1	61.9	.27	51.4	34.5	.67
7th. day	208.2	53.0	.25	47.2	31.0	.65

Table 53. Changes in the carbohydrate and moisture content of Stowell's Evergreen sweet corn.

Storage at 28° C.		Moisture	Reducing sugars as dextrose		Total sugars as invert sugar		Soluble polysaccharides (dextrins)		Starch	
period	Atm.		wet wt.	dry wt.	wet wt.	dry wt.	wet wt.	dry wt.	wet wt.	dry wt.
0	----	78.93	1.22	5.80	4.03	19.11	1.10	5.21	7.12	33.80
7 days	air	74.87	.74*	2.93*	1.30*	5.16*	2.54*	10.11*	8.40	33.43
7 days	N_2	81.86	2.10	11.58	2.29	12.61	2.03*	11.21*	3.56	18.14

* Figures result of a single determination. Others the average of duplicate samples.

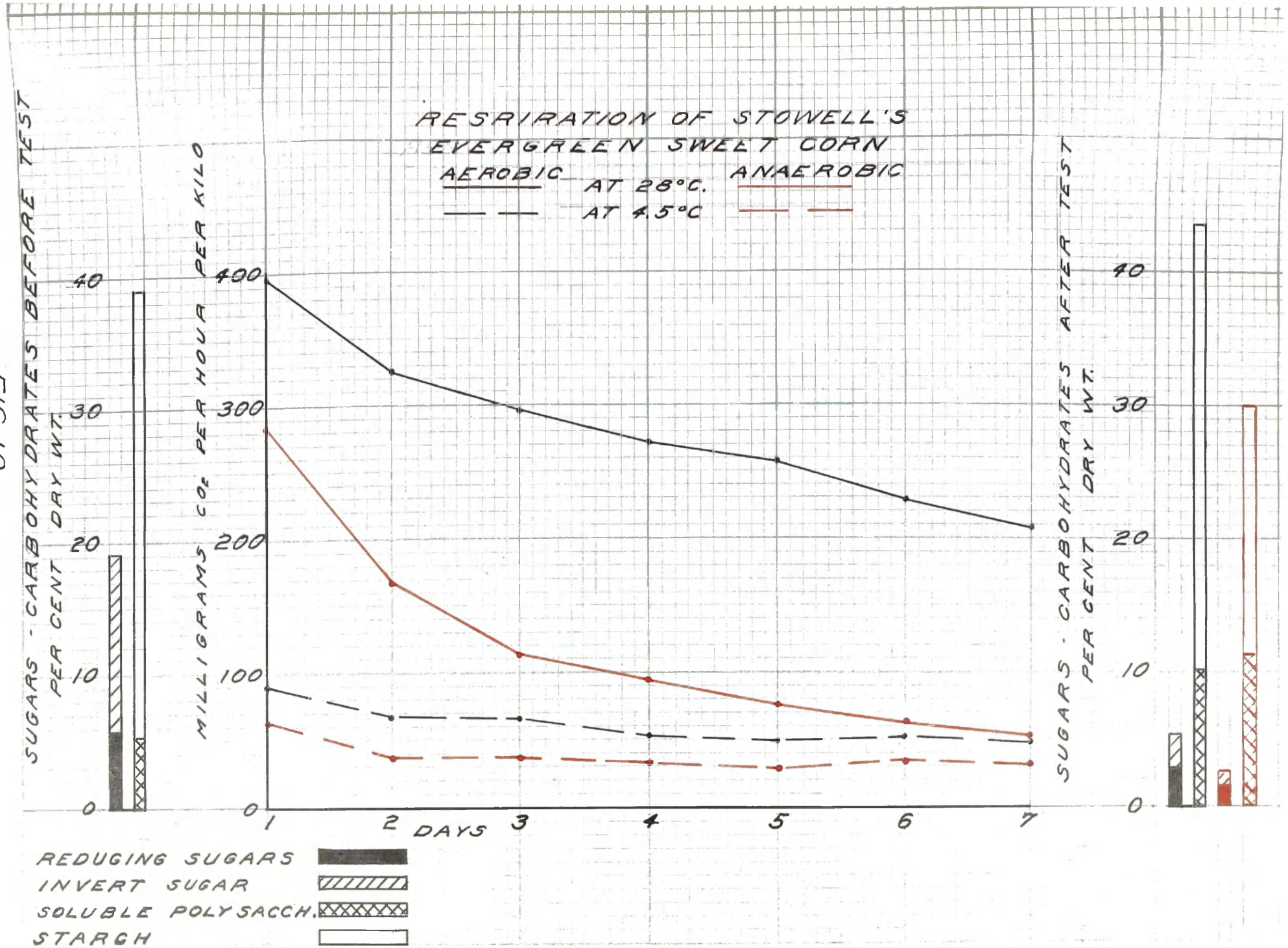

RESPIRATION OF STOWELL'S EVERGREEN SWEET CORN
AEROBIC
AT 28°C.
ANAEROBIC
AT 4.5°C
MILLIGRAMS CO_2 PER HOUR PER KILO
400
300
200
100
0
1
2
DAYS
3
4
5
6
7
SUGARS - CARBOHYDRATES BEFORE TEST
PER CENT DRY WT.
40
30
20
10
0
SUGARS - CARBOHYDRATES AFTER TEST
PER CENT DRY WT.
40
30
20
10
0
REDUCING SUGARS
INVERT SUGAR
SOLUBLE POLYSACCH.
STARCH

FIG. 10.

Discussion

The sweet corn had by far the highest respiratory rate of any material studied. The only work previously reported on sweet corn (Appleman, 1918) gives respiration values approximating one-tenth of the ones reported herein. Unpublished data by Dr. Parker shows respiratory rates very similar to the values reported.

The grains, whether milk stage or older, seemed to continue toward maturity in aerobic conditions. The surfaces of the grains shrunk and the endosperm became more doughy, and the moisture content decreased in most cases. Concurrent with the visible changes, the starch content of the grains increased, and the sugar content decreased. The samples of Truckers' corn (table 51) showed a decrease in water soluble polysaccharides (dextrins) but the Stowell's Evergreen (table 53) increased in this fraction. The latter variety was very low in soluble polysaccharides at the beginning of the experiment, and the second analysis seems to have been made while the sugars were only partially polymerized to starch.

In anaerobic conditions, however, the grains remained very fresh and plump in appearance. The juice of the kernels became less milky in appearance, and there was an apparent increase in the moisture content. It is not known whether this latter change was due to an increase in water from metabolic processes or to an increase in volatile compounds formed by the anaerobic process. The latter view is favored because alcohol was present, judged by odor, in the grains after a period of anaerobiosis. It is more reasonable to expect an increase in such products of anaerobic respiration than an increase in water.

An interesting fact is the pronounced increase of reducing sugars in the grains during anaerobiosis. The total sugar content was greater after

anaerobic than after aerobic respiration but was somewhat less than in the original sample. The Truckers' corn showed a decrease in soluble polysaccharides and starch during anaerobic respiration. The Stowell's Evergreen corn decreased much more in starch content, but the dextrins increased somewhat.

It is evident that the tendency in sweet corn is toward hydrolysis of the higher carbohydrates during anaerobic respiration; very little synthesis, or polymerization occurs. It is interesting to compare the carbohydrate changes in sweet corn with those of the pea, Pisiun sativum, in the germinating seeds of which, Nabokich (1903) reported that hydrolysis of starch took place very slowly in the absence of oxygen.

The Hopeland variety was the only one tested at different stages of maturity. There is no outstanding difference in the respiration of corn in the milk, or in the dough stage. The respiration is greater for the first few days in corn in the milk stage (tables 47 and 48).

The anaerobic respiration decreased more rapidly than the aerobic in all the experiments except that on corn in the dough stage. This results in a gradual decrease in the I/N values. At low temperatures, the anaerobic respiration does not decrease so rapidly and the I/N values are almost constant. In the experiment with Truckers' corn, when the samples were changed from a low to a warm temperature, the I/N value decreased steadily, after having remained almost constant in the cold. The decrease in anaerobic respiration is probably due to the inhibiting, or toxic, action of intermediate products of metabolism. Certainly, it is not due to a lack of fermentable food substance because the samples contained more sugars at the end of the respiration period than at the beginning.

Mature Corn and Soybeans

Dried grain of corn (Zea Mays L.) and dried seeds of soybean (Glycine Max Merr.) were used in the following experiments.

The experiments are designed to extend the work of Bailey (1921) who studied the respiration of corn at different moisture percentages, and of Bailey and Gurjar (1918) who determined the respiration of stored wheat. Bailey found that the respiration of corn increased rapidly with increase in the moisture content of the grain above 17 per cent. Bailey and Gurjar made one test of the anaerobic respiration of wheat and found the I/N value to be about 0.40 when the moisture content was about 16 per cent. Hill (1913) used wheat having a much higher moisture content, and found I/N values of about 0.5 in grain sterilized with alcohol, but only about .21 in grain sterilized with formalin. Diakonow (Kostychev, 1927) found that seeds of Ricinus communis, which are very low in carbohydrate content, had a very low anaerobic respiration.

The corn grain and soybeans used in these experiments were grown on the Maryland Experiment Station farm.

To secure material of different moisture content, distilled water was added directly to the material in sufficient quantity to raise the moisture content to the percentage desired. The material was stirred frequently for the first few hours to keep the water uniformly distributed over the surfaces of the grains or seeds until it had all been imbibed. Two respiration samples were weighed out of each lot of soaked material, thus insuring samples as nearly identical as possible. After adding the water, two or three days were allowed for it to distribute evenly throughout the internal tissue of the grain, or seed. During this time, the material

was stored in jars, loosely stoppered, so anaerobic conditions would not occur.

In all experiments with grains and seeds, the respiration is calculated on basis of dry material, and not on total weight of sample.

Experiment 1.-- The first experiment was on dried grain of Hopeland sweet corn. This grain was from sample ears saved from selection plots. Three rows of kernels were shelled from each ear; about 50 ears were represented, one-half being grown in 1931, the other in 1932. The total lot of grain weighed about 1400 grams. Broken kernels, and chaff were removed, the lot then subdivided and water added. Respiration samples weighed 500 grams each for corn of low moisture content, and 200 grams each for corn high in moisture. The preliminary period was about 17 hours. The gas flows were continuous throughout the respiration period except for a short stop each day while the copper in the nitrogen line was being reduced. See table 54.

Experiment 2.-- Tests were also run on Reids Yellow Dent corn. The corn was selected from the crib, only well matured ears being used. The kernels were discarded from tips and butts of ears. The first two tests were on corn grown in 1932, the last two on corn grown in 1933. The respiration samples were prepared in the same manner as those of sweet corn. The samples high in moisture weighed 250 grams; those low in moisture weighed 500 grams. The results are presented in table 54.

Table 54. Respiration studies on corn grain at 22° C.

Material	Date of exp.	Moisture	Carbon dioxide liberated per hour per kilo dry wt.		
			aerobic	anaerobic	I/N
		per cent	mg.	mg.	
Hopeland sweet corn	Nov. 30 - Dec. 3, 1932	15.11	4.71	1.25	.264
		20.58	22.99	12.39	.539
Reid's Yellow Dent, grown 1932	Dec. 20 - 23, 1932	17.38	5.12	1.48	.290
		22.09	27.83	16.24	.584
Reid's Yellow Dent, grown 1933	Dec. 5 - 8, 1933	14.69	2.02	.24	.120
		19.10	3.82	1.37	.357
	Mar. 8 - 11, 1934	16.38	1.77	.44	.250
		23.06	25.72	13.69	.532

Similar experiments were run with soaked seeds of the soybean, Virginia variety. The data are given in table 55.

Table 55. Respiration studies on soybeans at 22° C.

Source of Seed	Date of test	Moisture	Carbon dioxide liberated per hour per kilo dry wt.		
			aerobic	anaerobic	I/N
		per cent	mg.	mg.	
1932	April 3 - 6, 1933	13.12	.23	.18	.78
		19.09	2.43	.79	.32
1933	March 27 - 30, 1934	13.34	.42	.30	.71
		17.51	1.25	.48	.38
1933	April 12 - 15 1934	14.48	.45	.34	.74
		22.23	6.72	1.83	.27

Discussion

It seems from the experiments reported in tables 54 and 55 that there is some difference in the reaction of corn grain and soybeans to anaerobic conditions. Corn of low moisture content has a comparatively low anaerobic respiration; the I/N values are about 0.25 or even less. When the moisture content is increased, the anaerobic respiration becomes relatively greater, and the I/N values are about 0.50, with one exception, where the moisture content was below 20 per cent.

On the other hand, soybeans have a low I/N value when the moisture content is high. The high I/N values for soybeans at low moisture contents are considered possibly inaccurate. The respiration is at a very low rate, and if the correction for the blank in titration was too low, the high values would result. In one instance, air was drawn through an empty chamber for 72 hours, and the blank was higher than the blank through which air had not been passed. Using the higher blank the I/N values for the soybeans at high and low moisture contents were not significantly different. The data on soybeans support the results of Diakonow (Kostychev, 1927) who found very weak anaerobic respiration in seeds of Ricinus communis which are very low in carbohydrate.

GENERAL DISCUSSION

The purpose of this general discussion is to present a general survey of the results, and compare the response made by different materials to similar treatments.

In the experiments presented in this report, the periods of anaerobiosis extended for longer times than have been used by most previous workers. As previously explained, with thick storage organs, considerable time is necessary for the free oxygen in the tissues to be displaced or used up by oxidation. Not until the free oxygen is entirely absent in the tissue is anaerobiosis possible. Much early work reports the anaerobic respiration for short periods only, and the accuracy of such determinations is sometimes doubtful. When the anaerobic respiration is determined several days in succession, it is possible to ascertain from study of the data, whether or not true values are obtained. In some experiments, the value for the anaerobic respiration would be much higher for the first day than for the second. In such cases, it is considered that the preliminary period was not long enough to secure complete removal of the free oxygen in the tissue, and that a part of the carbon dioxide liberated during the first day came from oxidation reactions.

There is considerable difference in the length of time that different materials withstand anaerobic conditions without injury. Potatoes of the Irish Cobbler variety seldom showed injury after a period of six days without oxygen. McCormick potatoes, however, often showed considerable injury, ie. black, sunken areas on the surface of the tubers, after four days experience under anaerobic conditions. Carrots showed little or no injury after a week in the absence of oxygen. Parsnips were similar

to carrots in their response, but turnips indicated some injury at the end of three days. Tomato fruits withstood only three or four days anaerobiosis at a warm temperature before the tissue broke down, and the juice leaked out.

A study of the I/N ratios also shows that some materials withstand anaerobiosis better than others. In some, such as carrots, and parsnips, the I/N values remained more or less constant for several days, while with green sweet corn, the I/N ratios decreased steadily. A sharp increase in the I/N ratio, or of the anaerobic respiration after a period of anaerobiosis, usually indicates injury, and the rise is probably caused by autolytic actions.

The sugar content of potatoes seems to show some correlation with the rate of aerobic respiration. Potatoes were stored at three temperatures, 22° C., 6.5° C., and 2°C., approximately. Those stored at the low temperature accumulated the most sugar, and had the highest aerobic respiration when removed to a higher temperature. Those stored at 6.5° C. accumulated less sugar, and showed a less pronounced rise in the respiration. The tubers stored at 22° C. were used as controls. Parsnips accumulated some sugar during cold storage, but the respiration rate was not increased at subsequent high temperature.

The results obtained with potatoes and carrots after treatment with ethyl bromide differ from those of Morkowin (1903) who found that stimulants increase the aerobic and anaerobic respiration in the same proportions. Treatment with ethyl bromide resulted in greater stimulus to the aerobic than to the anaerobic respiration, both in potatoes and carrots.

Chudiakow (1894) found that the I/N values remained the same at different temperatures. With the potatoes used in this problem, the

I/N values decreased more rapidly at low temperatures than at high temperature. On the other hand, the I/N values of green sweet corn remained higher at the lower temperatures. The greater decrease of the I/N values at high temperature may be explained by the fact that toxic, or inhibiting, products of anaerobic respiration accumulate more rapidly at higher temperature and thus cause a more rapid decrease in the I/N values. No explanation is advanced for the case when the I/N values decrease more rapidly at the low temperature.

Smirnoff (Kostychev, 1927) found that wounding increased both types of respiration to the same degree. Lutman (1926) disagreed, finding that wounding did not stimulate the anaerobic respiration to the same extent as it did the aerobic. The experiments carried out in this work support Lutman's conclusions.

The initial increase in the respiration of potatoes after a period of cold storage is more pronounced in the aerobic than in the anaerobic respiration.

The theory of the genetic connection of the anaerobic and the aerobic phases of respiration is based in part on the fact that the two phases have been found to respond to the same stimuli in the same proportions. The results of some of the experiments mentioned above show that the two phases of respiration do not always respond in the same degree to certain stimuli. For this reason, some of the experimental results do not support the theory of the genetic connection of the anaerobic and aerobic phases of respiration.

SUMMARY

Exposure to ethyl bromide gas; storage in nitrogen; wounding; and a period of cold storage all resulted in a greater proportional increase in the aerobic respiration of potatoes than in the anaerobic phase.

The I/N values for potatoes tended to decrease more rapidly during respiration at a low temperature than at a high temperature.

The I/N values decreased more rapidly at high than at low temperatures in green sweet corn, carrots, parsnips, and tomatoes.

The aerobic respiration of onions did not show an initial rise after a period of cold storage, but was maintained at a high rate, whereas the aerobic respiration of unstored onions decreased steadily. The anaerobic respiration of onions was not stimulated by cold storage.

The sugar content of onions increased slightly during cold storage.

Tomato fruits at the pink stage had a higher aerobic respiration rate than green or red ripe fruits. Green fruits had the highest anaerobic respiration rate at 30° C., but the fruits in the pink stage had the highest rate at 5.5° C. Red ripe fruits had the lowest anaerobic rate at 30° C. and had about the same anaerobic rate at 5.5° C. as green fruit.

Carrots and parsnips both had comparatively high anaerobic respiration rates. At warm temperatures, carrots consistently liberated more carbon dioxide under anaerobic than under aerobic conditions.

Parsnips did not show a pronounced initial rise in respiration after a period of cold storage.

The sugar content of parsnips increased during cold storage.

Turnips did not have a high anaerobic respiration; the I/N values varied from .4 to .8. The turnips did not withstand anaerobic conditions well.

Green sweet corn had extremely high respiration rates. The anaerobic respiration was much less than the aerobic.

There seemed to be a tendency toward hydrolysis of the carbohydrates in sweet corn during anaerobic storage. Synthesis of higher polysaccharides occurred in aerobic conditions.

Mature corn at low moisture percentages had very low I/N values. As the moisture content was increased, the I/N values also increased, indicating a relative increase in the anaerobic respiration over the aerobic.

Soybeans had a very low anaerobic respiration at the moisture percentages used, 13 to 23 per cent.

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